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An Evaluation of the Use of Sulfur Dioxide in Fumigant Mixtures for Grain Treatment[1]

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Sulfur dioxide (SO_2) has been used as a constituent in liquid grain fumigants for a number of years. Many questions as to the usefulness of SO_2 in concentrations of 1 to 3% by weight in fumigant mixtures have arisen. Some questions have been raised whether SO_2 synergizes with other fumigants to increase insect kill, "freshens" moldy grain, cools grain, reduces the flash point of fumigants containing carbon bisulfide, and reduces the moisture content of the treated grain. An examination of these effects in laboratory tests has been conducted in order to assess the practical utility of SO_2 in grain fumigant mixtures. One major requirement of a good grain fumigant is that the fumigant (or mixture) must control all insect species and life stages in all depths and portions of all types of stored grain without damaging the grain. Some other important qualifications for a grain fumigant are lack of explosive properties, low toxicity to humans, minimal corrosiveness to application equipment and grain containers, and a reasonable cost of the fumigant and its application.

GRAIN SORPTION AND PENETRATION TESTS.—Since grain is the commodity being fumigated, it is important to know the sorption properties of SO_2 in grain. The degree of sorption in turn governs the fumigant concentration in the interstitial air spaces of the grain at any given time after application of the fumigant and is related to the insecticidal efficiency of the fumigant.

If a fumigant mixture containing 2% SO_2 by weight is applied at a dosage rate of 6 gallons per 1000 bushels of grain, the concentration of SO_2 in the interstitial air spaces of the grain does not usually exceed 1.5 pounds of SO_2 per 1000 cubic feet (M.c.f.). This assumes even distribution of the gas throughout the grain mass and assumes no sorption by the grain. The dosage of the fumigant is based on grain estimated to have about 40% air space. In order to determine the SO_2 sorption properties of grain a dosage of SO_2 was used in excess of that which is used commercially. Accordingly, a laboratory test was devised in which 30 pounds of 14.3% moisture corn was placed in a modified 25.5-liter aluminum pressure cooker (Heresite lined). The air in the cooker was evacuated and a theoretical dosage rate of 60 pounds of SO_2 per 1000 cubic feet (based on interstitial grain space) was introduced and the vacuum satisfied with room air. The cooker was shaken and rotated at frequent intervals during the first 5 minutes after introduction of the fumigant in order to assure an even distribution throughout the bulk of the corn.

Gaseous concentrations of SO_2 in the treated cooker were pumped continuously over a period of 24 hours from the cooker through a Gow-Mac thermal conductivity unit (TCU) and back into the cooker. The thermal conductivity unit was calibrated to measure the various concentrations of SO_2 in air. By use of the above technique the relative sorption of SO_2 by corn was determined by the residual concentration of SO_2 in the interstitial air spaces of the treated corn. TCU readings were taken at various time intervals during the 24-hour exposure period (fig. 1). The above test was repeated at three temperatures: 48° F., 68° F., and 88° F., and the results are shown in figure 2.

The highest dosages obtained at each temperature are far below the theoretical 60 pounds per 1000 cubic feet dosage applied. This is partially due to rapid sorption in the grain. The higher the temperature, the faster the decrease in the gaseous concentration of SO_2. It is known also that SO_2 reacts with moisture, bleaches pigments, and is generally unstable in the presence of fruit, vegetables and certain other commodities. For the purpose of confirming the sorption of SO_2 from the interstitial air spaces of treated corn, samples of this air were taken and analyzed by means of the mass spectrometer. At the end of 4 hours at 68° F., the thermoconductivity unit reading was 0.6 pounds per 1000 cubic feet compared to 0.9 by mass spectrometer analysis; likewise at 88 °F. the thermoconductivity reading was 0 versus 0.25 with the mass spectrometer. This again confirms the rapid sorption and probable reaction of SO_2 in grain. Based on practical dosages of SO_2 at 68 and 88° F., the concentration in air would be quickly reduced to sublethal quantities for insects. With such a rapid reduction in the gaseous concentrations in air, the question is posed as to whether the SO_2 in a grain fumigant mixture applied to the surface of grain stored in bins, as is done commercially, will be able to penetrate deeply into the grain mass before sorption and reaction takes place.

In order to answer the above question, the penetration properties of SO_2 in grain were studied by using two types of tools; that is, by means of insect mortality and by

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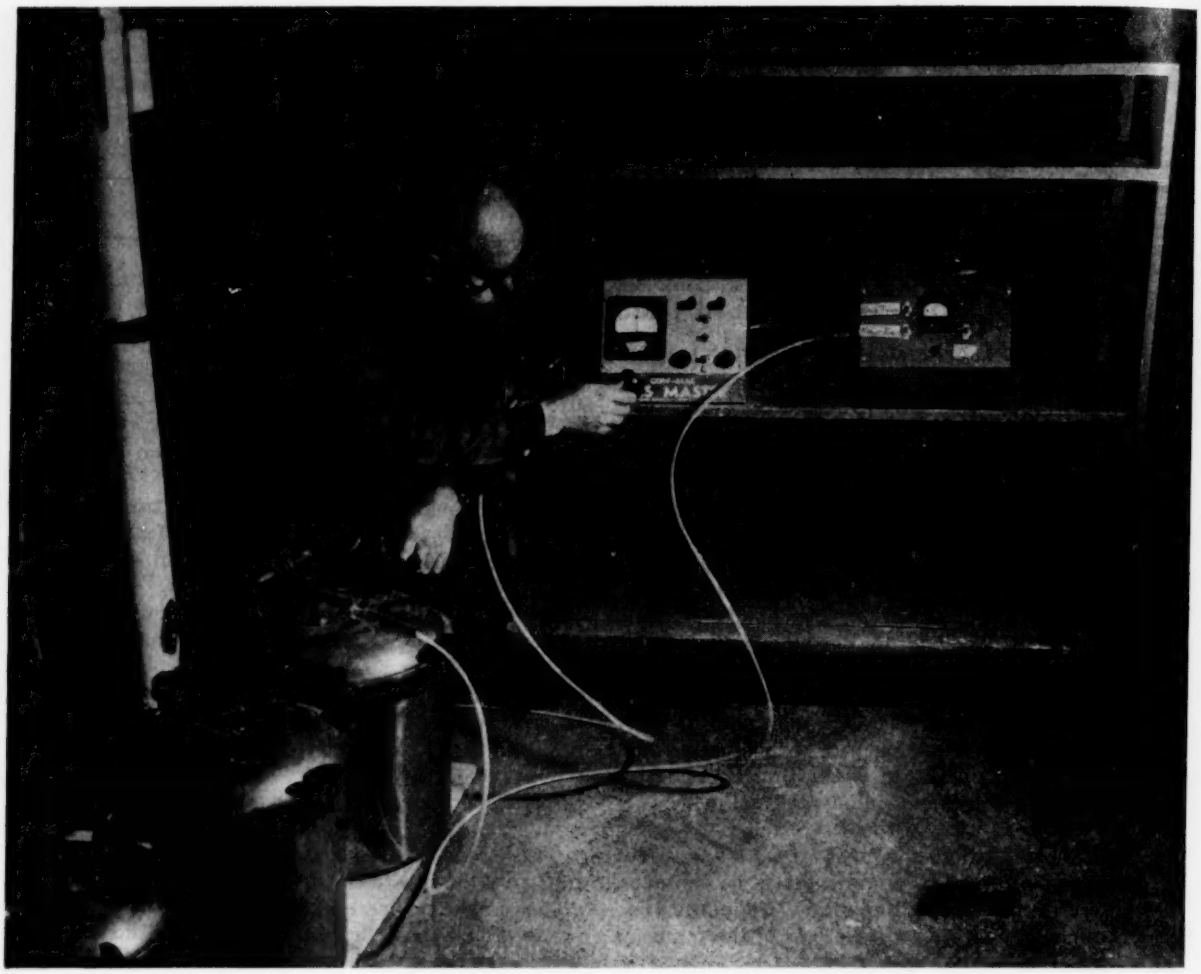

FIG. 1.—Checking fumigant concentration in grain sorption studies.

means of air samples analyzed by mass spectrometry. For the purpose of these tests, corrugated metal grain towers 6 feet tall and 9 inches in diameter were used (figure 3) which, when full, contained 2 bushels of grain. The fumigant was applied to the surface of the grain through a polyethylene film seal on top of the tower by means of a hypodermic syringe and needle. The gas was then allowed to penetrate downward by gravity and diffusion. The tower was essentially leakproof except for normal gaseous expansion and contraction around the top seal. Holes in the side of the tower were at the 0-, 3-, and 6-foot depths in the grain (surface, middle, and bottom). Through these holes the introduction and removal of insects and taking of air samples was permitted. These holes were closed with rubber stoppers except during the brief periods of time needed for taking the above samples.

In order to test the effect of various amounts of SO_2 in grain fumigants, a number of commercial fumigant mixtures were selected and tested with and without SO_2. The compositions of the fumigant mixtures used are shown in table 1.

The insecticidal effect of the above mixtures was determined by the use of two insect species of widely divergent susceptibility to fumigants; namely, larvae of the black-carpet beetle *Attagenus piceus* (Oliv.) and adults of the confused flour beetle *Tribolium confusum* Duv. Successive groups of these insects were confined and exposed at the surface, middle and bottom of the grain tower for intervals of 3 days, twice a week for 18 days after application of the fumigant. Mortality counts were made 2 weeks after the beginning of the exposure period. The total number of days following application of the fumigant wherein wheat retained enough toxicant to kill at least 90% of both insect species was used as the criterion of residual effectiveness. Penetration effects of the mixture were analyzed by studying the insecticidal effects at the surface of the grain (top) and the 6-foot depth (bottom). These tests were conducted with wheat having a moisture content of 13 to 14% and a temperature of 48° F. was used because the least sorption and reaction takes place at this temperature as shown in figure 2. The degree of penetration into stored grain by the fumigant mixtures listed in table 1, based on the residual effectiveness against the two insect species is shown in table 2. These tests show no indication that the addition of SO_2 was beneficial at any depth in the grain.

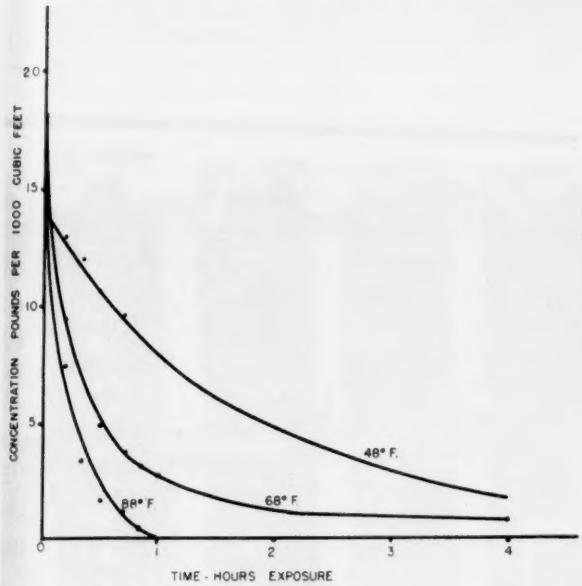

FIG. 2.—The relative sorption of SO_2 in corn at various temperatures (as measured by concentrations in interstitial air).

The speed of penetration of stored grain was tested by using the Dowfume 75[2] plus 7.5% SO_2 and "80–20" plus 2% SO_2 compositions shown in table 1. These two fumigant mixtures were applied under the same conditions as the previously mentioned residual insecticide tests; however, in this case, mass spectrometry analyses of air samples were used to determine speed and degree of penetration in wheat of the components of the fumigant composition. Interstitial air samples at several depths in the wheat were taken 2 to 3 hours after the application of the fumigants as shown in table 3. It will be noted that the surface was the only place where SO_2 was found to be present, and this in small quantities only.

Based on the gas laws in which the low molecular weight gases diffuse more rapidly than higher molecular weight gases, SO_2 should disperse in all directions more rapidly than carbon tetrachloride or ethylene dichloride. Due to sorption and reaction, such was not the case in grain fumigation; in fact, just the opposite was true. In the Dowfume 75 test, the peak concentration of CCl_4 was at the 1.5-foot depth and had not yet reached the 4.5-foot depth at the end of 2 hours. The peak concentation of ethylene dichloride was still at the surface, as was SO_2. If the SO_2 were penetrating the grain without appreciable sorption, the peak concentration of gas would probably be at the 3- to 6-foot depth at the time these air samples were taken. In the "80–20" test, CCl_4 and CS_2 reached the 3-foot depth in high concentrations. Here, again, no SO_2 was present.

The typical pattern of penetration of wheat with grain fumigant mixtures shows penetrating ingredients reaching a given depth in a high concentration "wave" or "front," and then passing on, much as it would in a chromatographic separation column. No such "wave" or "front" was shown for SO_2 in these tests.

In another type of penetration test, Kenaga (1953) showed that it took 2.4 times as much CCl_4 to obtain an LD^{100} using confused flour beetle adults in a 10-inch depth of flour as it did in space (16 hours exposure at 80° F.). Under the same conditions it takes three times as much CS_2. In more recent tests, under similar conditions it was shown that it takes more than 12 times as much SO_2 to penetrate *5-inches* of flour to obtain the LD^{100}.

Table 1.—Some liquid grain fumigant compositions with and without SO_2.

Fumigant Mixture	Composition—Per Cent by Weight					
	EDB[a]	CCl_4	CS_2	EDC[b]	SO_2	Other
Dowfume 75	—	29.8	—	70.2	—	
Dowfume 75+2% SO_2	—	29.2	—	68.8	2.0	
Dowfume 75+7.5% SO_2	—	27.6	—	64.9	7.5	
Dowfume EB-5	7.2	63.6	—	29.2	—	
Dowfume EB-5+1% SO_2	7.1	63.0	—	28.9	1.0	
Dowfume EB-5+2% SO_2	7.1	62.3	—	28.6	2.0	
CCl_4	—	100.0	—	—	—	
CCl_4+2% SO_2	—	98.0	—	—	2.0	
"80-20"	—	83.5	16.5	—	—	
"80-20"+2% SO_2	—	81.8	16.2	—	2.0	
Composition No. 1	4.8	27.4	—	64.7	3.1	
Composition No. 2	1.2	83.8	—	—	3.0	Benzene 12.0 (by analysis)

[a] EDB = Ethylene dibromide
[b] EDC = Ethylene dichloride

Table 2.—Residual insect mortality resulting from fumigant mixtures in different depths in wheat at 48° F.

Fumigant Mixture 4 Gals. per 1000 Bu.	Number of Days After Application 90% of Insects Killed			
	Black Carpet Beetle Larva		Confused Flour Beetle Adult	
	Depth in Grain (Ft.)		Depth in Grain (Ft.)	
	0	6	0	6
Dowfume 75	3	0	*18*[a]	0
Dowfume 75+2% SO_2	3	0	*18*	0
Dowfume 75+7.5% SO_2	3	0	*18*	0
Dowfume EB-5	11	0	*18*	*18*
Dowfume EB-5+1% SO_2	7	0	*18*	*18*
Dowfume EB-5+2% SO_2	7	0	*18*	*18*
CCl_4	3	0	14	*18*
CCl_4+2% SO_2	3	0	7	*18*
80-20	3	3	11	*18*
80-20+2% SO_2	3	3	3	*18*
Composition No. 1	3	0	*18*	0
Composition No. 2	3	0	*11*	3

[a] *18* = 100% control at end of 18 days.

Table 3.—Penetration of SO_2-containing grain fumigants into wheat at 48° F. based on mass spectrometer analyses.

Fumigant Mixture 4 Gallons per 1000 Bu.	Hours After Application	Position in Wheat	Lbs./1,000 Cubic Feet[a]			
			CCl_4	CS_2	EDC[b]	SO_2
Dowfume 75+7.5% SO_2	2	surface	3.9	—	5.5	0.02
Dowfume 75+7.5% SO_2	2	1.5 ft. deep	4.7	—	1.7	0
Dowfume 75+7.5% SO_2	2	3 ft. deep	0.2	—	0.2	0
Dowfume 75+7.5% SO_2	2	4.5 ft. deep	0	—	0	0
Dowfume 75+7.5% SO_2	2	6 ft. deep (bottom)	0	—	0	0
"80-20"+2% SO_2	3	3 ft. deep	12.0	1.7	—	0

[a] Interseed air.
[b] EDC = Ethylene dichloride.

[2] A product of Dow Chemical Co. containing ethylene dichloride, 75%, and carbon tetrachloride, 25% (by volume).

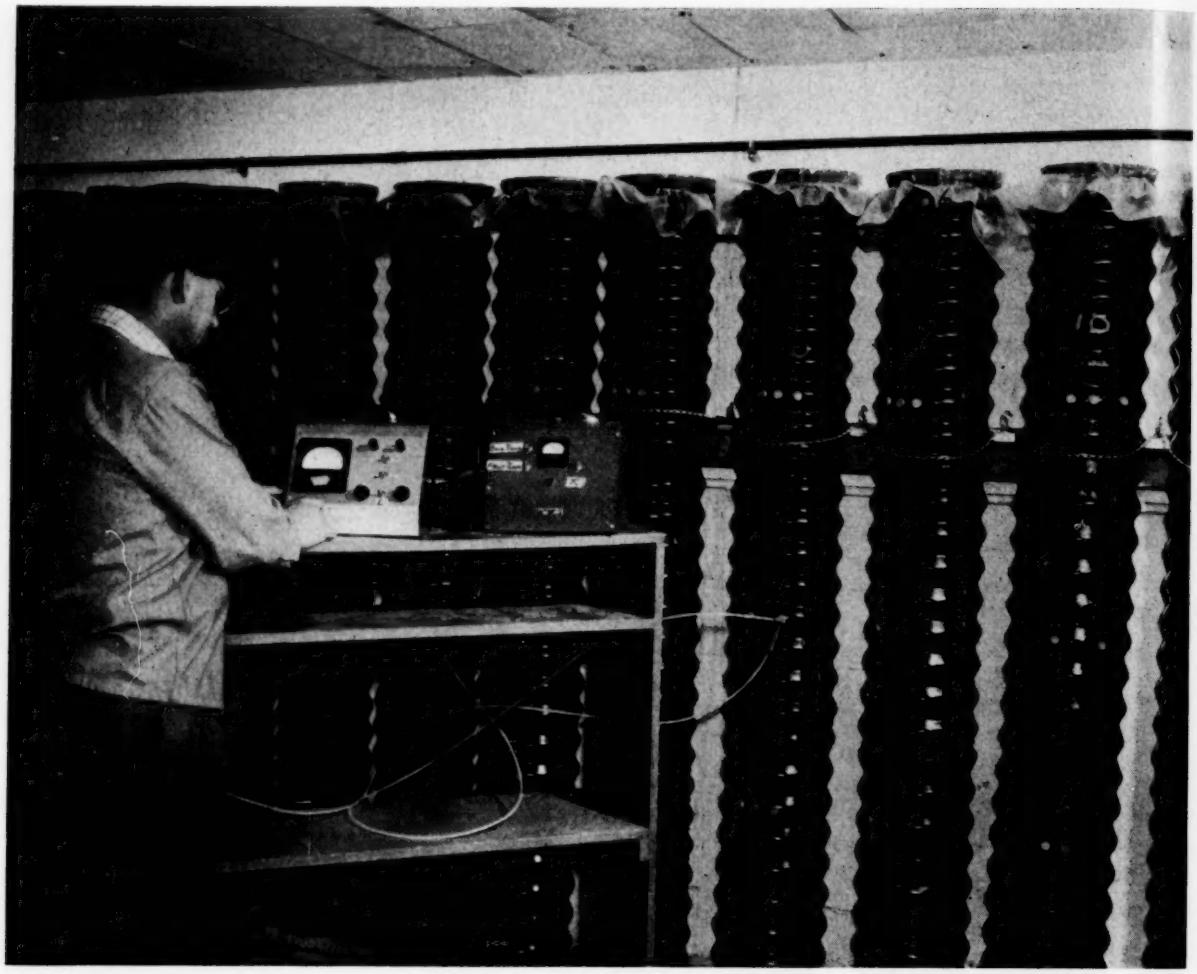

FIG. 3.—Checking fumigant concentration in grain penetration studies.

Further evidence supporting the lack of satisfactory penetration of SO_2 can be shown from insecticidal data obtained during the course of the first 24 hours of the residual tests shown in table 2. These 24-hour insecticidal data are shown in table 4 and indicate no appreciable effect of added amounts of SO_2 in aiding the insecticidal properties at the 3- and 6-foot levels. A difference of $\pm 15\%$ in mortality in this type of test is usually not significant. These data do not exclude increased insecticidal properties in the top portion of the grain.

Table 4.—Penetration of grain fumigants in wheat at 48° F. based on insect mortality during the first day of exposure.

Fumigant Mixture 4 Gallons per 1000 Bu.	Per Cent Mortality—24-Hour Exposure					
	Black Carpet Beetle Larva			Confused Flour Beetle Adult		
	Depth in Grain (Ft.)			Depth in Grain (Ft.)		
	0	3	6	0	3	6
Dowfume 75+2% SO_2	100	0	0	100	94	0
Dowfume 75+7.5% SO_2	100	15	0	100	97	0
EB-5	100	30	0	100	100	0
EB-5+1% SO_2	100	27	0	100	100	0
EB-5+2% SO_2	100	55	0	100	100	9
"80-20"	100	76	15	100	100	73
"80-20"+2% SO_2	100	76	27	100	100	76

Dennis (1951) has shown the increased insecticidal activity of "80–20" mixture when 2% SO_2 is added. The increase in toxicity in space is understandable due to the superior insecticidal toxicity of SO_2 over CS_2 and CCl_4. However, the data showing increased activity of the SO_2 in "80–20" mixture over "80–20" alone in grain treatments is not supported by the evidence given here. Apparently SO_2 is highly sorbed under these conditions. A reaction of SO_2 with the grain (or moisture contained therein) could explain the poor penetration and the fact that it has no residual insecticidal value in fumigant mixtures.

SHORT TERM SPACE FUMIGATION TESTS WITH SO_2.—Sulfur dioxide may have short term toxic effects on insects located on the surface of grain in storage bins. In view of the rapid disappearance of SO_2 from the interstitial air in grain, SO_2 would need to kill insects quickly if it were to be useful, even near the place where the fumigant was applied. It is recognized that SO_2 may act synergistically or additively with other ingredients in

the fumigant mixture; however, very little is known about the toxicity of SO_2 alone when used for short periods of exposure to insects. A number of investigators including Shepard *et al.* (1937), Busvine (1938), and Swisher (1944) have reported on the effects of a 5-hour exposure of SO_2 to some *adult* grain insects (table 5). It was thought desirable to determine the LD^{50} and LD^{95} of several insect species and different life stages at 80° F. for short exposure periods (i.e., 0.5, 1, and 5 hours). The insects used included confused flour beetle adults (2 to 4 weeks old) and eggs (2 days old), black carpet beetle larvae (4 months old), and granary weevil adults (1 to 2 weeks old), *Sitophilus granarius* (L.). The insects were confined in slotted plastic vials which were in turn placed in modified 25.5-liter pressure cookers. The cookers were closed, evacuated, and the SO_2 applied by measuring the gas through a mercury-filled gas burette. After the desired dosage was applied, the vacuum in the cooker was equilibrated to atmospheric pressure and held for the desired exposure period. The insects were held for 2 weeks after fumigation before the final mortality counts were made. Each dosage mortality exposure was triplicated with 33 insects of each species in each replicate and plotted on logarithmic probability scale paper. A line was drawn to fit the dosage mortality points from which the LD^{50} and LD^{95} were calculated. These data are shown in table 5. It is apparent that SO_2 is quite toxic to egg, larval, and adult life stages of these grain infesting beetles. It is possible to obtain high concentrations of SO_2 in the first stages of the treatment of bin-stored grain when liquid fumigant mixtures containing SO_2 are applied in the conventional manner to the top area of the grain in the bin. On this basis, it is likely that SO_2 will assist in killing surface infesting insects which are present at the time of the applications.

EFFECT OF SO_2 IN GRAIN FUMIGANTS APPLIED TO MUSTY GRAIN.—One of the characteristics attributed to fumigant mixtures containing SO_2 is that it freshens grain and removes the musty odor of moldy grain. In order to test this property, Composition No. 2, (see table 1) was chosen as a representative of a grain fumigant containing SO_2. Dowfume EB-5[3] and Dowfume 75 were chosen for comparison as fumigant mixtures containing no SO_2. Both musty and fresh corn in gallon jars were treated in duplicate tests with these fumigant mixtures. The treated samples were exposed to the fumigant for a week and then aired for several weeks.

Table 5.—Toxicity of SO_2 to several grain insect species and life stages at 25° C. using various exposure periods.

Insect Life Stage	Hours Exposure	Dosage, Mg. per L. LD^{50}	Dosage, Mg. per L. LD^{95}	Reference
Black carpet beetle larva	5	11.2	16.0	Kenaga
	1	16.5	23.2	
	0.5	25.6	35.2	
Confused flour beetle egg	5	7.8	9.1	Kenaga
	1	18.9	25.6	
	0.5	39.2	53.6	
Confused flour beetle adult	5	6.8	8.0	Kenaga
	1	7.8	9.9	
	0.5	9.8	12.2	
Granary weevil adult	5	5.4	6.7	Kenaga
	1	11.5	13.0	
	0.5	12.2	13.8	
Confused flour beetle adult	5	5.6	—	Swisher
Confused flour beetle adult	5	5.7	10.7 (LD^{99})	Shepard *et al.*
Rice weevil adult	5	17.0	46.9 (LD^{99})	Shepard *et al.*
Granary weevil adult	5	5.7	11.3 (LD^{99})	Shepard *et al.*
Rice weevil adult	5	—	10.8 (LD^{100})	Busvine (20° C.)
Red flour beetle adult	5	—	9.7 (LD^{100})	Busvine (20° C.)
Granary weevil adult	5	—	8.3 (LD^{100})	Busvine (20° C.)

Table 6.—Effect of liquid grain fumigants with and without SO_2 on masking musty grain odors.

Grain Condition	Material Applied	Dosage, Gals./1000 bu.	Matchings: Correct	Matchings: Incorrect	Does Treatment Mask Musty Odor?
Musty Fresh	Comp.[a] No. 2 Comp. No. 2	4 4	6	0	no
Musty Musty	Comp. No. 2 none	4 none	1	5	no
Musty Fresh	Comp. No. 2 none	4 none	6	1	no
Musty Musty	Comp. No. 2 Comp. No. 2	4 30	3	3	no
Musty Fresh	Dowfume EB-5 Dowfume EB-5	4 4	6	0	no
Musty Musty	Dowfume EB-5 none	4 none	0	6	no
Musty Fresh	Dowfume EB-5 none	4 none	6	0	no
Musty Musty	Dowfume EB-5 Dowfume EB-5	4 30	5	1	no
Musty Musty	Dowfume EB-5 Comp. No. 2	30 30	2	4	no
Musty Musty	Dowfume 75 Comp. No. 2	4 4	2	4	no

[a] Comp. = Composition.

A panel of six people was organized to see if odor differences between treatments were detectable. Each panelist went through a series of 10 triangular determinations in which duplicate samples of one treatment were to be matched against a single sample of another treatment. Each panelist was asked to match the duplicate samples. The results of these tests are shown in table 6. If the duplicate samples were matched correctly, it is recorded as one correct in the table, otherwise it was marked incorrect. The results show that even with such heavy dosages as 30 gallons of Composition No. 2 per 1,000 bushels, the musty odor was not eliminated. The composition containing SO_2 was no better than Dowfume EB-5 and Dowfume 75 in eliminating musty odor after airing the grain. The difference between musty and fresh grain was always detectable, irrespective of the fumigant treatment. There appeared to be a detectable difference between the 30-gallon and 4-gallon dosage of EB-5 on musty grain, probably due to some residual ethylene dibromide odor; however, neither sample was acceptable because of the residual musty odor.

SO_2 may temporarily desensitize a person's olefactory organs. In the presence of SO_2, the musty odor may not be detectable in fumigated musty grain which has not been thoroughly aired of fumigant vapors. Even carbon tetrachloride vapors will help to mask a musty odor under such conditions. It is concluded that none of the fumigant mixtures tested, including the one containing SO_2,

[3] A product of Dow Chemical Co. containing 7.2% ethylene dibromide, 29.5% propylene dichloride, and 63.6% carbon tetrachloride.

have the effect of changing the odor of musty grain permanently.

EFFECTS OF GRAIN FUMIGANTS CONTAINING SO_2 ON THE TEMPERATURE OF TREATED GRAIN.—There are two ways in which a fumigant might act to cool grain; one being the indirect effect of killing insects and other organisms attacking the grain and the other a direct effect by evaporation of the fumigant applied as a liquid to the grain. It is assumed that the fumigant will kill insects and possibly certain fungi, bacteria, yeast, etc. thereby eliminating "hot spots" of infestation and infection; otherwise it would not do a successful job. A grain fumigant is expected to do this indirect type of cooling.

The type of cooling caused by the evaporation of grain fumigants is shown in table 7. These tests were conducted in the 6-foot grain towers. The fumigants were poured on the top surface of the grain. Dosages, grains, temperature and air currents were varied. Under the most extreme conditions of evaporation (78° F. using corn with a strong air current passed over the grain surface) an excessive dosage of 8 gallons of fumigant per 1,000 bushels of grain lowered the temperature only 13° F. on the surface, 4° F. at a 4-inch depth and 1° F. at a 12-inch depth. The temperature of the bottom 5 feet of grain was not changed. At cold temperatures and with no evaporative air movement, no significant reduction of temperature was noticed where SO_2 was used with the grain fumigants. The data in table 7 show no significant temperature changes of a practical value due to the use of the SO_2. As a matter of fact temperature changes of a similar magnitude occur on the grain surface because of normal atmospheric temperature changes. The cooling effect by fumigant evaporation does not reach the critical infested "hot spots" located deep in the grain mass, and therefore is of no significant value there either.

EFFECT OF GRAIN FUMIGANTS CONTAINING SO_2 IN REDUCING THE MOISTURE CONTENT OF THE GRAIN.—Two grain fumigants, Composition No. 2 and Dowfume 75, were applied to the surface of 78° F. wheat contained in gallon jars, at the dosage rate of 4 gallons per 1,000 bushels. The top of the jar was left open in a dry room (40 to 60% relative humidity) after the application to permit immediate volatilization. Most of the fumigant was vaporized from the jar by the end of 18 hours. The moisture content of the grain was determined with a Steinlite Moisture Tester. Grain was obtained from the surface and bottom of the grain mass at different intervals of time after fumigation and the moisture content compared with that occurring at the beginning of the test. Results, shown in table 8, indicate that the untreated check lost as much or more moisture than any of the treatments.

Dowfume 75+7.5% SO_2 applied in a 6-foot corrugated tower, using wheat at 48° F., showed no decrease in moisture at the end of 5 days in samples taken from the surface of the grain (see table 8). The use of grain fumigant mixtures, with or without SO_2, does not appear to have affected appreciably the moisture content of grain.

EFFECT OF SO_2 ON THE FLAMMABILITY OF FUMIGANT MIXTURES CONTAINING CS_2.—Comparison of the halo, flash and fire points of the "80–20" fumigant mixture alone and with the addition of 2% or 5% SO_2 is shown in table 9.

With flammable mixtures such as 80–20 the halo, flash, and fire point can be depressed somewhat; however, "80–20" cannot be made completely non-flammable with the addition of 2 to 5% SO_2. It is to be pointed out that the low-boiling SO_2 is rapidly lost from the mixture upon exposure to air and any reduction in fire hazard is temporary.

The flash point improvement resulting from the addition of SO_2 as shown in table 9 may apply to practical conditions such as hazards encountered when a new can of fumigant is first opened, and also during the application of the fumigant to the grain. These results do not

Table 7.—Effect of surface applications of fumigants containing SO_2 on the grain temperature.

Fumigant Applied	Grain	Dosage, Gals./M. Bu.	Inches Depth in Grain	Temp. ° F.	Minutes After Application	Air Current Over Top
Dowfume 75 +7.5% SO_2	wheat	0	0[a]	47	start	none
	wheat	0	18	47	start	none
	wheat	0	36	47	start	none
	wheat	4	0	47	1	none
	wheat	4	0	47	5	none
	wheat	4	0	47	30	none
	corn	0	0	78	start	none
	corn	4	0	74	10	none
Mixture of Dowfume 75 +7.5% SO_2 and Composition No. 2	corn	4+4	0	65	25	strong
	corn	4+4	0	74	30	strong
	corn	4+4	4	74	25	strong
	corn	4+4	8	76	25	strong
	corn	4+4	12	77	25	strong
	corn	4+4	18	78	25	strong
	corn	4+4	0	77	120	strong
	corn	4+4	18	78	100	strong

[a] 0 = Surface of grain.

Table 8.—Effect of grain fumigants on the moisture content of wheat.

Fumigant Mixture[a]	Per Cent Moisture Before Treatment	Per Cent Moisture Content After Treatment		Time After Treatment	Test Chamber	Temp., ° F.
		Surface	Bottom			
Dowfume EB-5	14.2	14.2	—	25 minutes	gallon jar	78
		14.0	14.2	18 hours		
Composition No. 2	14.1	14.1	—	23 minutes	gallon jar	78
		13.8	14.0	18 hours		
None (check)	14.4	13.4	14.0	18 hours	gallon jar	78
Dowfume 75 +7.5% SO_2	14.5	14.7	—	5 days	6-foot tower	48

[a] Dosage was 4 gallons per 1000 bushels.

Table 9.—The effect of SO_2 on the flammability of fumigant mixtures containing SO_2.

Fumigant Mixture	Cleveland Open Cup, °F.		
	Halo Point[a]	Flash Point[b]	Fire Point[c]
80-20 (80% CCl_4+20% CS_2 by vol.)	—	9	9?
80-20+2% SO_2	−15	25	75
80-20+5% SO_2	− 5	35	70

[a] Halo point = no flash or fire, supports combustion around flame; "halo" effect around flame.

[b] Flash point = flash of vapor, fire goes out.

[c] Fire point = flash of vapor + liquid surface burns.

apply to hazards encountered where a source of flame or spark is present below the upper few feet in the grain mass or around the outside of the grain bin. Since SO_2 is quickly sorbed in the upper layers, it would be of no value in suppressing flammable ratios of CS_2 which penetrate into the grain mass.

SUMMARY.—The use of sulfur dioxide in liquid fumigant mixtures for the treatment of grain has been studied under laboratory conditions. By means of insecticidal evaluation and mass spectrometry analyses of air samples it was shown that SO_2 was highly sorbed and/or reacted in grain. Under the conditions of these tests where the fumigant mixtures were applied to the surface of the grain mass and the grain was contained in an essentially leakproof chamber, SO_2 did not have an appreciable penetration effect below the surface of the grain. This combination of properties restricts the usefulness of SO_2 in grain fumigant mixtures to grain nearest the point of application of the fumigant and even these uses are temporary in nature. Under these conditions sulfur dioxide would *temporarily* safen the initial use of flammable grain fumigants, mask the musty odor of spoiled grain until fumigant vapors are aired, and enhance the kill of insects on the surface areas of grain immediately after application. The use of SO_2 in grain fumigant mixtures did not appreciably affect residual insect kill. affect the moisture content of the grain, eliminate musty odor in grain, cool the grain mass or safen the use of flammable CS_2-containing grain fumigants which penetrate the major portions of the grain.

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The Synthesis of C^{14}-Labeled Piperonyl Butoxide and Its Fate in the Madeira Roach[1]

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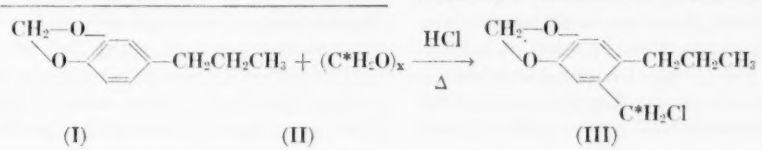
Although current concepts of pyrethrum synergism are summarized in reviews by Blackith (1953), Schmidt (1955) and Kearns (1956), the exact relationship between pyrethrum and a synergist such as piperonyl butoxide is not known. Piperonyl butoxide, one of the more potent pyrethrum synergists, was developed by Wachs (1947). A knowledge of the individual actions of synergist and toxicant would appear to be useful for a better interpretation of their combined action. The synthesis of C^{14}-labeled piperonyl butoxide, α-[2-(*n*-butoxyethoxy)-ethoxy]-4,5-methylenedioxy-2-propyltoluene-α-C^{14}, was undertaken as an aid in studying the mode of action and metabolism of this compound in insects and mammals.

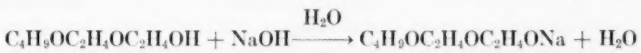
MATERIALS AND METHODS.—*Radioactive preparation.*—Three reactions are involved in the synthesis. In step 1, dihydrosafrole (I) is reacted with paraformaldehyde (II) in the presence of HCl, fortified by bubbling dry HCl through it, to form the chloromethyl derivative (III) of dihydrosafrole. The sodium salt of diethylene glycol *n*-butyl ether (IV) was prepared as shown in step 2 and the products of the first two steps condensed (step 3) to yield piperonyl butoxide (V) (* indicates C^{14}).

Step 1.



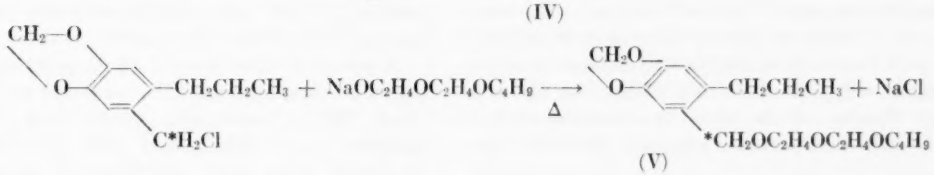
(I) (II) (III)

Step 2.



(IV)

Step 3.



(V)

PAID PAPER.

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Several microsyntheses were made with non-radioactive materials. Dihydrosafrole (2.0 gm.; 0.012M) and paraformaldehyde (0.45 gm.; 0.015M) were added to fortified HCl (3.2 gm.). This mixture was shaken continuously and heated slowly to 80° C. over a period of 2 hours. The final temperature was maintained and the shaking continued for an additional 4 hours. A small glass reaction vessel was designed with standard taper 10/30 openings at the top for a thermometer and venting tube, and a glass-stoppered opening at the bottom to permit easy withdrawal of compound III. The lower half of the reaction vessel was jacketed with an electric heating mantle. At the end of the reaction period, the lower layer (crude compound III) was drawn off and centrifuged. Any aqueous supernatant fluid was siphoned off and discarded. The purity of the product (III) was checked by determining its saponification number.

The sodium salt of diethylene glycol *n*-butyl ether was prepared by adding diethylene glycol *n*-butyl ether (5 gm.; 0.031M) to a warm, aqueous NaOH solution (0.6 ml. 25M NaOH). While the contents were stirred magnetically, the flask was evacuated and warmed until all the water from the caustic solution as well as that produced in the reaction was distilled off. The chloromethyl compound then was added through a dropping funnel. The reaction was allowed to proceed with constant stirring at 118–120° C. for 2 hours. During this period sodium chloride crystals were formed. The flask was allowed to cool and distilled water (2.7 ml.) was added to dissolve the salt and excess alkali. The mixture was shaken thoroughly and centrifuged. The brown, supernatant liquid contained the crude piperonyl butoxide; the lower brine layer was discarded. Vacuum distillation was employed to remove not only the excess diethylene glycol *n*-butyl ether but also to obtain pure piperonyl butoxide.

A dilute radioactive synthesis was made by adding 300 μg. of C^{14}-labeled paraformaldehyde, prepared according to the method of Murray *et al.* (1952) and having an activity of approximately 1 mc. per millimole,[3] to non-radioactive paraformaldehyde. For the main radioactive synthesis, 0.03 gm. of the C^{14}-labeled paraformaldehyde was added to 0.43 gm. of non-radioactive paraformaldehyde and the preparation carried out as outlined previously.

Analytical Methods.—The ultraviolet absorption spectrum of the radioactive piperonyl butoxide, purified by vacuum distillation, was compared to a pure, non-radioactive standard. Infrared spectra[4] were determined for a pure piperonyl butoxide standard, the radioactive piperonyl butoxide, dihydrosafrole and diethylene glycol *n*-butyl ether. Spectra of the latter two compounds were prepared to help interpret the piperonyl butoxide spectra.

The refractive indices of certain distillation fractions, pure piperonyl butoxide and diethylene glycol *n*-butyl ether were determined on an Abbe, Model 56, refractometer.

The distilled piperonyl butoxide was analyzed by the colorimetric procedure of Jones *et al.* (1952). Later in the investigation the colorimetric method of Blum (1955b) was used exclusively as it proved to be more sensitive than the other method. Blum (1955b) modified the Labat (1933) test by substituting acetonitrile and a 2-hour color development period for the ethyl alcohol and a boiling water bath. Because solvents other than acetonitrile, such as acetone and carbon tetrachloride, greatly increased the color intensity, the role of solvents in this colorimetric test was investigated.

The products of the radioactive synthesis were counted using either a windowless, gas-flow counter, Nuclear Model D-46A,[5] or a thin window (1.9 mg./cm.^{2}) Geiger-Mueller tube connected to a Berkeley Decimal Scaler.[6] A C^{14}-labeled polystyrene standard, with a counting rate of approximately 4400 cpm. in the windowless counter was used as a reference source. All samples were prepared in duplicate on $1\frac{1}{4}$"-diameter aluminum planchets and counted long enough to attain a standard error of $\pm 5\%$ (Calvin *et al.* 1949).

The determination of activity of all radioactive test solutions used in chromatographic and biological investigations was made using a manual sample changer, Model M-5,[5] and a Model D-47[5] gas-flow counter equipped with a "micromil" window.

A chromatographic method of analysis for piperonyl butoxide has apparently not been reported. Of the many different solvent systems tried for the resolution of piperonyl butoxide on paper chromatograms, acetonitrile-methanol-water 2:2:1 (v/v), suggested by Blum (1956) gave the best results.

Paper chromatograms containing radioactive piperonyl butoxide were scanned with a suitably shielded Geiger-Mueller tube fitted with a "micromil" window (120 μg./cm.^{2}). The tube rested on an aluminum base plate into which a groove, 1" wide and $\frac{1}{4}$" deep, had been machined to accommodate the paper strips. The area of the strip exposed to the counting tube was varied by means of two adjustable steel plates located between the "micromil" window and the moving strip. Chromatograms were taped to the chart of an automatic recorder[7] actuated by a Nuclear Model 1620 rate meter.[5] The chromatograms were scanned at the rate of 6 inches per hour with a 1 cm. slit width and with the rate meter set at the 300 cpm. (counts per minute) range.

The strong absorption band of piperonyl butoxide at 290 mμ. indicated that another method for the detection of this compound on paper chromatograms was possible. An adapter which fits into the absorption cell compartment of a Beckman, Model DU, spectrophotometer (Tennent *et al.* 1951) was used for scanning paper chromatograms in the ultraviolet region.

A visual method for detecting piperonyl butoxide on paper chromatograms was also developed. Two coal tar dyes, D&C Violet #2, 1-hydroxy-4-*p*-toluino-anthraquinone, and D&C Red #18, 1-zylylazoxylylazo-2-naphthol, were tried. Solutions of these dyes, 0.05% (w/v) in *n*-hexane were used to spray paper chromatograms. Beroza (1955) suggested that concentrated sul-

[3] Obtained from Isotopes Specialties Co., Glendale, Calif.

[4] The infrared analyses were made by R. A. McCord of the Institute for Atomic Research, Iowa State College, Ames.

[5] Obtained from Nuclear Instrument and Chemical Corporation, Chicago, Ill.

[6] Model 2105 Decimal Scaler, Berkeley Scientific Division of Beckman Instruments Inc., Richmond, Calif.

[7] Model AW, The Esterline-Angus Co., Inc., Indianapolis, Ind.

furic acid might be used for the detection of methylenedioxyphenyl groups on paper chromatograms. A thin streak of concentrated sulfuric acid was pipetted onto a 9×55 cm. glass plate set in a wooden frame. The paper strip was then carefully placed over the acid. The glass plate was turned over and the location of the reddish areas, indicating the presence of methylenedioxyphenyl groups, was marked on the glass plate with a wax pencil.

EXPERIMENTS WITH THE MADEIRA ROACH.—Thirty-five male and 35 female adult Madeira roaches, *Leucophaea maderae* (F.) were used to study the absorption of radioactive piperonyl butoxide. These cockroaches were treated under carbon dioxide anesthesia, with 80 μg. of radioactive piperonyl butoxide on the ventral thoracic area. The cockroaches were provided with food (apple slices) and held at a temperature of 29±1° C. At 24 hour intervals after treatment, 5 males and 5 females were immobilized with carbon dioxide and rinsed with three 100 ml. portions of acetone. The rinses were combined, concentrated and evaluated radiometrically.

The absorption of piperonyl butoxide through the cuticle of dead cockroaches was also studied. Fifteen females were killed with hydrogen cyanide and then treated topically with radioactive piperonyl butoxide in the same manner as described above. At 24, 84 and 120 hours following treatment, 5 cockroaches were rinsed with acetone and the radioactivity present in the washes was evaluated.

To determine the rate of excretion of radioactive piperonyl butoxide, 30 male and 30 female adult cockroaches received the same dosage in the same manner as described above in the absorption experiment. Groups of 5 were placed in cylindrical, one pint cardboard cartons fitted at both ends with ¼″ mesh hardware cloth. The cartons were placed over petri dishes for collection of feces. The fecal pellets were collected daily, weighed and transferred into tightly closed 1-oz. glass bottles and held at about −15° C. until analyzed. The extraction procedure for feces consisted of adding 10 ml. of an 80% solution of acetone in water to the bottles containing the feces and soaking the pellets for 48 hours and then decanting the liquid and adding 10 ml. of fresh extraction solution. The extracts were combined, filtered through glass wool, concentrated and evaluated radiometrically.

To determine the amount of radioactive piperonyl butoxide present in the fecal extracts, the acetone was evaporated and the aqueous material remaining was extracted with carbon tetrachloride, and the latter extract evaluated radiometrically and chromatographically. In one experiment a petroleum ether extract was also chromatographed. In all cases, a chromatogram of a similar extract from control feces, containing 16 μg. of C^{14}-labeled piperonyl butoxide, was run with the experimental chromatograms.

In one of two tissue distribution experiments, 10 female cockroaches were treated topically with 41 μg. of radioactive piperonyl butoxide in the manner described previously. Twenty-four hours after treatment the cockroaches were anesthetized, rinsed with acetone, weighed and dissected. The antennae, wings and legs were removed and placed in a tared aluminum boat. To reduce evaporation, the weighing boats with the dissected material were kept in petri dishes containing a piece of moistened cotton. A dorsal longitudinal incision was made extending from the posterior end through the head capsule; the head capsule and body were pinned open and the following tissues removed: the digestive tract including fore-gut, mid-gut and hind-gut with Malpighian tubules, fat bodies, and brain plus thoracic ganglia. The remaining parts of the insect were added to the excised antennae, wings and legs and constituted what has been named the "remainder." The tissues from the 10 cockroaches were pooled, weighed and held at about −15° C. until they could be ground with sand and anhydrous sodium sulfate and extracted three times with petroleum ether.

In the other tissue distribution experiment, 5 females were treated topically in the same manner as above with 88 μg. of radioactive piperonyl butoxide. This group was sacrificed 48 hours after treatment and dissected as described previously. The feces from this group were also collected and analyzed.

EXPERIMENTAL RESULTS.—*Synthesis of Piperonyl Butoxide.*—The purity of the chloromethyl derivative of dihydrosafrole (III) was indicated by determining its saponification number. In a series of eight non-radioactive runs the saponification number varied from 244 to 268 with an average of 258. An average yield of 2.2 ml. of the chloromethyl compound was obtained from eight runs. In a typical run, 3.18 gm. (0.0094M; 77% yield) of colorless piperonyl butoxide was obtained by vacuum distillation, b.p. 155° C. (0.3 mm. Hg), and n_D^{25} 1.4985 as compared to n_D^{25} 1.4991 for a reference sample. Wachs (1947) reported the boiling point as 180° C. (4 mm. Hg).

In the dilute radioactive synthesis, the aqueous layer was discarded after the preparation of compound III since it contained less than 0.5% of the original radioactivity. No radioactivity was found in the excess diethylene glycol *n*-butyl ether collected during the initial phase of vacuum distillation. The balance of the activity was divided among the dark brown, viscous residue remaining in the distillation flask and the piperonyl butoxide coating the inside of the distilling apparatus.

The radioactive piperonyl butoxide from the main synthesis, purified by vacuum distillation, weighed 3.18 gm. and had the following properties: b.p. 160° C. (0.5 mm. Hg), n_D^{25} 1.4993, and 98% purity as determined colorimetrically. The over-all yield was 61% based on C^{14}-labeled paraformaldehyde. The radioactive piperonyl butoxide had a counting rate of 155 cpm./μg. as measured on a Nuclear Model D-46A gas-flow counter and a calculated activity of 0.157 μc./mg.

The ultraviolet absorption spectra of the radioactive product and a pure standard sample are shown in figure 1. The points of maximum absorption, 238 mμ. and 290 mμ., were the same for both samples. The differences in optical density are due to slight differences in molar concentrations of the two solutions. The two samples also were subjected to infrared analysis and appeared to have essentially identical absorption spectra (fig. 2). From the infrared spectra of dihydrosafrole and diethylene glycol *n*-butyl ether, certain bands of the absorption spectrum of piperonyl butoxide can be ascribed to functional groups as follows: The absorption at 1090 $cm.^{-1}$ (A) is due to the ether linkages of the diethylene glycol *n*-butyl ether side chain, and the two bands at either

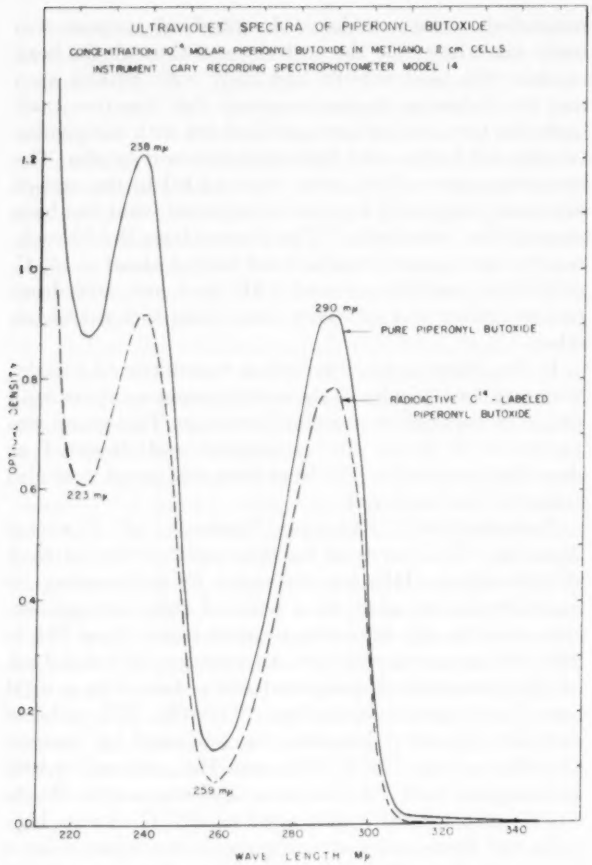

FIG. 1.—Ultraviolet spectra of piperonyl butoxide.

side of it, 1250 cm.$^{-1}$ (B) and 1035 cm.$^{-1}$ (C), are probably contributed by the methylenedioxy group. Some of the phenyl ring vibrations are reflected in the band at 1480 cm.$^{-1}$ (D) and a smaller one at 1610 cm.$^{-1}$ (E). The 1360 cm.$^{-1}$ band (F) is due to C—H bending contributed mainly from the *n*-propyl side chain. The strong absorption at 2850 cm.$^{-1}$ (G) results from C—H stretching vibrations. Miller *et al.* (1952) reported some infrared absorption bands for piperonyl butoxide but did not identify the bands with functional groups.

Effects of Solvents on the Colorimetric Test of Blum (1955b).—By accident, acetone was used instead of acetonitrile as the solvent in the Blum test. The resulting solution was a much darker green than the comparable acetonitrile samples. An acetone blank failed to give a green color. It was subsequently shown that even a small amount of acetone in experimental samples tested by this colorimetric procedure would give erroneous results. Absorption spectra of the color complexes obtained with piperonyl butoxide and the following solvents: acetone, carbon tetrachloride, methyl ethyl ketone, *n*-hexane and acetonitrile were compared. The general shapes of the spectral transmittance curves were similar but considerable variation in color intensity occurred with the different solvents. Very little color development resulted with *n*-hexane. Maximum absorption for the acetonitrile complex was at 670 mμ. as compared to 660 mμ. reported by Blum (1955b). The peaks obtained with the other solvents were close to 665 mμ.

A comparison of the colorimetric methods reported by Jones *et al.* (1952) and Blum (1955b) for piperonyl butoxide, using five organic solvents is shown in figure 3. The optical density of reagent blanks was always low, e.g., less than 0.01. Purified kerosene is the solvent rec-

FIG. 2.—Infrared spectra of piperonyl butoxide.

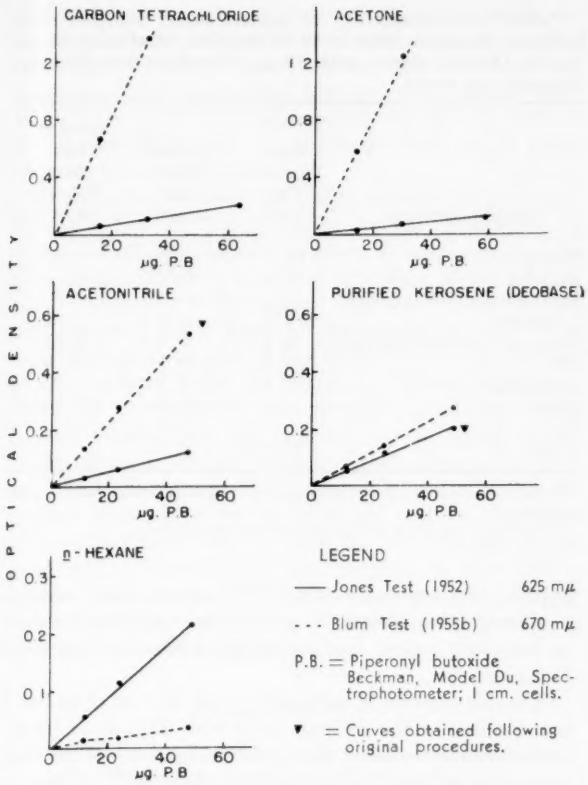

FIG. 3.—A comparison of standard curves obtained with piperonyl butoxide and five organic solvents by the colorimetric techniques of Jones *et al.* (1952) and Blum (1955b).

ommended by Jones *et al.* (1952) and acetonitrile was used by Blum (1955b). Greater sensitivity was obtained with the Blum test and all solvents except *n*-hexane. The use of acetone and acetonitrile as solvents decreased the sensitivity of the Jones test. Several of these solvents may be used in extracting residues of piperonyl butoxide; great care should be taken to remove traces of the extracting solvents before conducting colorimetric analyses, especially in the case of the Blum test.

The effect of three other solvents, Skelly B, benzene, and methyl ethyl ketone on the Blum test was also investigated. The curve obtained with Skelly B was similar to *n*-hexane; the benzene curve resembled the one obtained with purified kerosene and the slope of the methyl ethyl ketone curve was midway between those obtained with acetone and acetonitrile.

Chromatographic Analysis of Piperonyl Butoxide.—The R_f values of radioactive piperonyl butoxide obtained with the acetonitrile-methanol-water solvent system, using descending chromatography and determined by radiometric and spectrophotometric scanning techniques on the same chromatograms, were 0.874 ± 0.009 and 0.872 ± 0.004, respectively. The close agreement between the two methods of analysis indicated that the same compound was being measured in both cases. The R_f value for pure, non-radioactive piperonyl butoxide, as determined spectrophotometrically, was 0.871 ± 0.007.

Two dyes, D&C Violet #2 and D&C Red #18 were used to spray chromatograms. As little as 5 µg. of piperonyl butoxide were made visible by the use of either of these dyes on test strips. On actual chromatograms these dyes were useful only with concentrations of piperonyl butoxide above 50 µg. The R_f values obtained by the use of these dyes agreed closely with those derived from radiometric evaluation.

The colorimetric procedures of Jones *et al.* (1952) and Blum (1955b) could not be adapted to paper chromatography since the phosphoric and sulfuric acids charred the filter paper strips. Beroza's (1955) technique got around this problem of immediate charring. A few moments after the test strip was placed on the glass plate streaked with concentrated sulfuric acid, reddish spots indicated the presence of the piperonyl butoxide. On test strips, 10 µg. spots were clearly visible and 2 µg. spots were faintly visible. When this technique was tested on actual chromatograms, it was impossible to detect spots containing 25 µg. or less of piperonyl butoxide, A further objection to this method is that it results in the total loss of the chromatograms.

Absorption and Excretion of Piperonyl Butoxide.—The absorption of radioactive piperonyl butoxide in female cockroaches over a 7-day period, following topical application of 80 µg. per cockroach, is presented in figure 4. The piperonyl butoxide was almost completely absorbed after 1 week. A similar curve was obtained with male cockroaches. Penetration of radioactive piperonyl butoxide also occurred through the cuticle of dead females (fig. 4), although at a slower rate than found in live insects.

The excretion of radioactivity in female cockroaches treated topically with 80 µg. of radioactive piperonyl butoxide per insect (equivalent to 35.8 µg./g. body

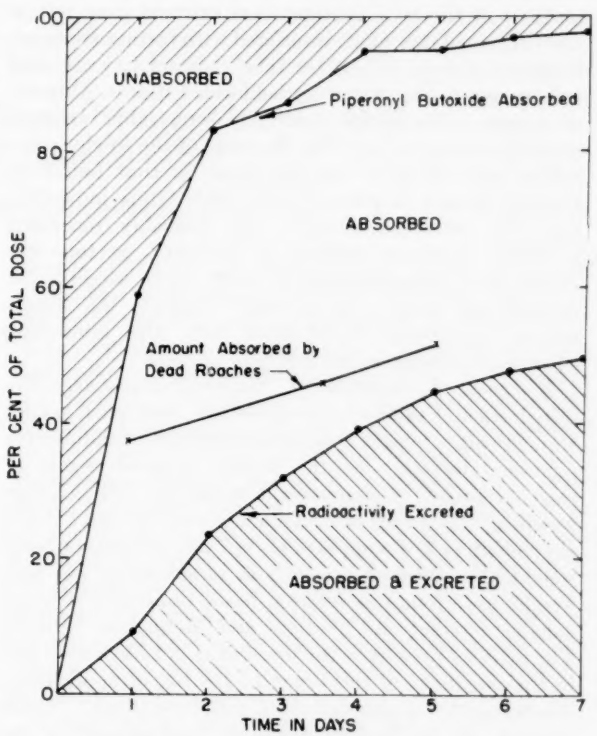

FIG. 4.—Absorption and excretion of radioactivity in female cockroaches treated topically with 80 µg. radioactive piperonyl butoxide per cockroach.

Table 1.—Radioactivity found in feces collected from 30 male and 30 female Madeira roaches treated topically with radioactive piperonyl butoxide (80 μg./insect).

Days Following Treatment	Males		Females	
	Weight of Feces (Mg.)	Activity (Cpm./Mg.)	Weight of Feces (Mg.)	Activity (Cpm./Mg.)
1	169	249.7	226	102.2
2	246	186.2	251	137.7
3	233	104.7	256	76.8
4	230	55.1	244	65.4
5	246	31.7	253	47.2
6	223	26.1	254	22.9
7	192	20.1	224	16.0
Total	1539	673.6	1708	468.2

weight) is also seen in figure 4. The rate of excretion in the male cockroaches was a little more rapid than in the females; this is apparent from the radioactivity excreted per mg. of feces listed in table 1. The smaller mean weight of the male cock roaches (1.55 g. vs. 2.23 g. for females) resulted in a greater dosage of piperonyl butoxide per unit of weight for the males. This might explain the more rapid excretion of radioactivity by the males.

The extraction procedure for cockroach feces is still not entirely satisfactory. Solvents such as diethyl ether and acetone, were tried but 80% acetone in water proved to be the most effective, because of water-soluble metabolites in the feces. Even with this solvent, some radioactivity remained in the feces after soaking for 5 days.

The carbon tetrachloride extracts of the aqueous portions of the 80% acetone fecal extracts were yellow and contained fats which interfered with further analyses. Slightly less than 50% of the total radioactivity excreted was present in the carbon tetrachloride extracts. Aliquots of carbon tetrachloride and petroleum ether extracts were chromatographed. The R_f value of the single radioactive spot obtained was the same as that found on chromatograms of piperonyl butoxide, thus indicating that the extracted material was probably piperonyl butoxide. Aqueous portions of the 80% acetone extracts were chromatographed with little success. No method was found which would separate the water-soluble radioactive metabolites from the interfering substances present in the aqueous extracts.

Biological Distribution of Piperonyl Butoxide.—The distribution of radioactivity in female cockroach tissues 48 hours after topical application of 88 μg. of radioactive piperonyl butoxide on the ventral thoracic region of each cockroach is summarized in table 2. The average weight of this group of cockroaches was 2.40 gm.; this is equivalent to a dosage rate of 36.7 μg. per gm. of body weight. The greatest amounts of radioactivity per unit weight were found as follows: feces, brain and thoracic ganglia, fore-gut and hind-gut and Malpighian tubules. The activity present in the fore-gut might have been due to the proximity of this organ to the site of application. The fore-gut was also distended with food (apples) which accounts for its disproportionately large amount of the total body weight. The radioactivity in the rinse (table 2) was calculated on the basis of the piperonyl butoxide

Table 2.—Radioactivity of pooled tissue samples from 5 female Madeira roaches 48 hours after treatment on the ventral thoracic region with 88 μg. of radioactive piperonyl butoxide per insect.

Sample Assayed	Body Weight (Per Cent)	Applied Dose (Per Cent)	Activity per Unit Weight (Cpm./Gm.)
Fore-gut	15.0	2.1	521
Mid-gut	5.3	0.1	79
Hind-gut and Malpighian tubules	2.4	0.3	465
Brain and thoracic ganglia	0.2	0.2	2,430
Fat	4.1	0.1	55
Remainder	65.1	2.1	122
Feces	—	47.9	258,500
Rinse	—	19.3	—
Total	92.1[a]	72.1[b]	—

[a] Total does not equal 100% because of weight losses in dissection and weighing.
[b] Total does not equal 100% because of extraction losses.

applied, although only 85% of the applied dose could be recovered from groups of cockroaches rinsed immediately following topical application of radioactive piperonyl butoxide.

The distribution of radioactivity at the end of 24 hours was similar to the 48-hour data with all values for internal recoveries being about half as large while the rinse was twice as large indicating that about 48% of the applied dose had been absorbed after 24 hours.

These results are in agreement with the findings of Blum (1955a) wherein 25% of 20 μg. piperonyl butoxide injected in the American cockroach, *Periplaneta americana* (L.), was found in the feces after 48 hours. On the other hand, he was able to recover 50% of topically applied piperonyl butoxide (20 μg. per cockroach) from the fat bodies and 20% from the central nerve cord 6 days after application; only small amounts were recovered from the feces.

Summary.—As an aid to studying the mode of action of piperonyl butoxide, one of the important pyrethrum synergists, radioactive piperonyl butoxide, α-[2-(2-*n*-butoxyethoxy)-ethoxy]-4,5-methylenedioxy-2-propyltoluene-α-C^{14}, was prepared by reacting C^{14}-labeled paraformaldehyde with dihydrosafrole and then condensing the resulting chloromethyl derivative of dihydrosafrole with the sodium salt of diethylene glycol *n*-butyl ether. The radioactive piperonyl butoxide was purified by vacuum distillation (over-all yield was 61% based on C^{14}-labeled paraformaldehyde) and had a calculated activity of 0.157 μc./mg. Ultraviolet and infrared spectra were used to establish purity and structure of the compound.

Three procedures for locating piperonyl butoxide on paper chromatograms, developed with acetonitrile-methanol-water as the solvent system, are described in addition to a radioactive scanning technique. Two dyes and a sulfuric acid streaking technique can be used on chromatograms containing over 50 μg. of piperonyl butoxide. For smaller amounts, an ultraviolet scanning method based on absorption at 290 mμ. was useful. The effect of organic solvents on two colorimetric tests (Blum

1955b and Jones *et al.* 1952) for piperonyl butoxide was investigated. Acetone, carbon tetrachloride, and methyl ethyl ketone produced more intense color complexes and *n*-hexane, Skelly B, purified kerosene, and benzene gave less intense colors than acetonitrile, the solvent used in the original Blum test. These solvents had only a slight effect on the Jones test.

The absorption and excretion of radioactive piperonyl butoxide was studied following topical application to male and female Madeira roaches, *Leucophaea maderae* (F.). About 88% was absorbed in 3 days; about 50% of the radioactivity in the applied dose was recovered from the feces in 7 days. Paper chromatographic analysis of fecal extracts showed that less than 50% of the radioactivity was from piperonyl butoxide, the remainder consisted of unidentified, water-soluble metabolites. The internal distribution of radioactivity in female cockroaches showed that the brain and thoracic ganglia, foregut, and hind-gut and Malpighian tubules contained the greatest amounts of radioactivity per unit weight. Since little radioactivity was found in the other tissues of the cockroach, it is postulated that the nervous tissue, foregut and hind-gut and Malpighian tubules are involved in the breakdown of radioactive piperonyl butoxide in the female Madeira roach.

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An Analysis of Twenty Years of Honey Bee Colony Weight Changes[1]

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The information given herein, although incomplete in some instances, is presented because it contains some significant data regarding the changes in weight of a honey bee colony on a scale over a period of 20 years, 1930 to 1948, inclusive, and 1955. The deficiencies in the records are indicated in the legend of table 1 and the lack of lines for parts of certain years in figure 1. The record is of a colony in the University of Illinois 40- to 50-colony apiary at Champaign-Urbana, Illinois. The colony was maintained in a 1½-story modified Dadant hive for wintering and managed for extracted honey production using a partial modified Demaree system. The apiary is located in the heart of the two cities at the edge of the University of Illinois campus which provided some open expanse for white Dutch clover which is mowed frequently. The two cities are traversed by two railroad right-of-ways, one approximately 0.5 mile distant and the other 0.7 mile away. The former provides some sweet clover pasturage within a mile radius while sweet clover in any quantity along the latter is beyond 1.5 miles. The bounds of the city are approximately 1 mile north and south, and a variable 2 or more miles east and west. While there is apparently some surplus flow from sweet clover, the bulk of the crop probably comes from white Dutch clover blooming abundantly in lawns of city homes and the larger areas of the University grounds, especially in years of frequent rains during the blooming period. There is some evidence that a part of the flow in some years may have come from soy bean fields a mile or more away. Honeydew apparently added to the flow, or at least prevented or reduced the loss of weight, during July and August of some dry years. In one year in the early 50's, not included in the records, at least a super of honeydew was stored by some colonies. It is apparent that beekeepers are not always aware of this fact, especially when bees are kept within city areas where aphids may be abundant on elm and oak trees.

HONEYFLOWS.—An attempt has been made to divide

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the year into three periods as shown in table 1; namely, spring, summer, and fall, listing the low and high points of each of these periods. The lowest weight of each year is considered as the zero or base point and is so depicted in figure 1 for all years except 1942, where the minimum colony weight dropped considerably below the normal spring or summer low after a swarm around June 6. It is evident that one cannot say that the changes in weight are always changes in the weight of stores. In the late fall and early winter or after brood rearing has ceased, on the average about October 15, changes in weight are due to loss of old bees and to reduction in stores. As brood rearing starts and stops during January and February and continues unabated during the spring months, then loss of weight of honey is in part replaced by weight of bees and brood, thus the recorded weight changes do not give a true picture of actual disappearance of vital stores. During the honeyflows, especially that of summer, the weight changes again probably are more indicative of added stores or the actual gain of honey in the hive as surplus. This is most likely to be true if the colony is somewhat crowded in the brood area and especially where a queen excluder is used at the beginning of the honeyflow to reduce the brood area to a single body. In the long run, it seems reasonable to assume that the total of the gains from spring to fall, inclusive, less the losses during the winter and spring and the two periods between honeyflows, actually represents the approximate surplus honey from year to year. Thus the average annual production from this scale colony has been approximately 85 pounds for the 20-year period.

Since the actual gains and losses in colony weight are clearly shown in figure 1 and table 1, the reader is referred to the latter for a general summary for the 20 years. From the information presented, it is apparent that even though the records are quite variable from year to year, the keeping of such records can give a beekeeper valuable information as to colony conditions which will enable him

Table 1.—Twenty years' weight changes[a] of a honey bee colony at Champaign-Urbana, Illinois. 1930–1948, 1955.

	Spring				Summer				Fall			
	Low		High		Low		High		Low		High	
Year	Date	Loss	Date	Gain	Date	Loss	Date	Gain	Date	Loss	Date	Gain
1930	—[b]	—	—	—	May 31	—	July 27	39	Aug. 25	6	Sept. 8	3
1931	Apr. 2	27	—	—	July 2	3	18	16	24	10	—	—
1932	6	—	—	—	June 8	6	19	114	10	4	—	—
1933	15	24	June 3	—[c]	5	4	10	58	11	+2	13	60
1934	28	64	May 22	23	2	2	23	126	6	2	Aug. 13	16
1935	11	53	11	3	11	22	Aug. 6	92	28	13	Sept. 19	23
1936	23	63	12	27	May 21	9	July 7	130	July 22	7	Aug. 9	14
1937	21	41	11	2	June 4	7	Aug. 5	49	—	—	Sept. 11	24
1938	Mar. 24	24	Apr. 18	—	May 15	7	2	284	Sept. 3	11	11	17
1939	Apr. 29	39	May 16	39	24	3	3	178	Aug. 24[d]	10	11	−10
1940	May 6	41	21	37	June 7	9	July 29	230	Sept. 3	20	20[e]	19
1941	Mar. 14[e]	29	10	38	17	32	Aug. 11	160	Aug. 24	11	10[f]	10
1942	Apr. 23[f]	26	Apr. 30	14	May 25	10	June 10	15	26	22	Aug. 31	10
1943	—	—	15	—	June 1	12	July 19	57	July 28	2	4	7
1944	Apr. 4	37	May 18	25	2	4	12	80	22	2	16	46
1945	Mar. 26	58	Apr. 19	12	May 28	26	Aug. 6	160	Aug. 20	8	Sept. 15	37
1946	Apr. 13[g]	41	25	6	29[h]	30	July 20	107	July 29	5	Aug. 5	5
1947	24	61	May 17	33	31	8	18[i]	14	Aug. 2	8	Sept. 24	57
1948[j]	22	42	15	27	27	7	June 20	42	8	12	7	−2
1955	9[k]	29	3	13	31	12	July 12[j]	145	—	—	Nov. 1	−46[l]
Variations:												
	Loss		*Gain*		*Loss*		*Gain*		*Loss*		*Gain*	
Dates	Mar. 14–May 6		Apr. 19–May 22		May 15–June 17		June 10–Aug. 11		July 22–Sept. 3		Aug. 4–Sept. 24	
Pounds	24 to 63		2 to 39		2 to 32		14 to 284		−2 to 22		−10 to 60	
Average[m]	−40 (17)		+21 (14)		−11 (19)		+105 (20)		−10 (19)		+20 (17)	

[a] The pounds of gain or loss indicated in each column are the differences between the dates of that column and the next preceding column, except the date preceding the first spring date is the one listed in the last column for the preceding year. For example, the 1930–1931 winter loss of 27 pounds is for the period from September 8, 1930 to April 2, 1931.

Variations and discrepancies in the records are indicated as follows:

[b] Incomplete records.

[c] Colony queenless April 15, 1933; new colony substituted June 3.

[d] No fall flow.

[e] Colony upset in windstorm, badly demoralized, winter loss recorded from October 7.

[f] Apparently partly robbed after examination September 10, plus errors in records; winter loss from February 18 to April 23 only.

[g] Winter loss from December 6 only.

[h] Same on June 7.

[i] Colony queenless June 4, 1947; requeened, may have swarmed in a good honey year.

[j] 1948, a year of very small surpluses, the total loss from June 20 to November 24 being 26 pounds.

[k] Colony established on scales, December 30, 1954.

[l] No fall flow.

[m] Figures in parentheses are numbers of years included in averages, i.e., those with complete records for the respective periods; the cumulative annual average was 85 pounds of surplus.

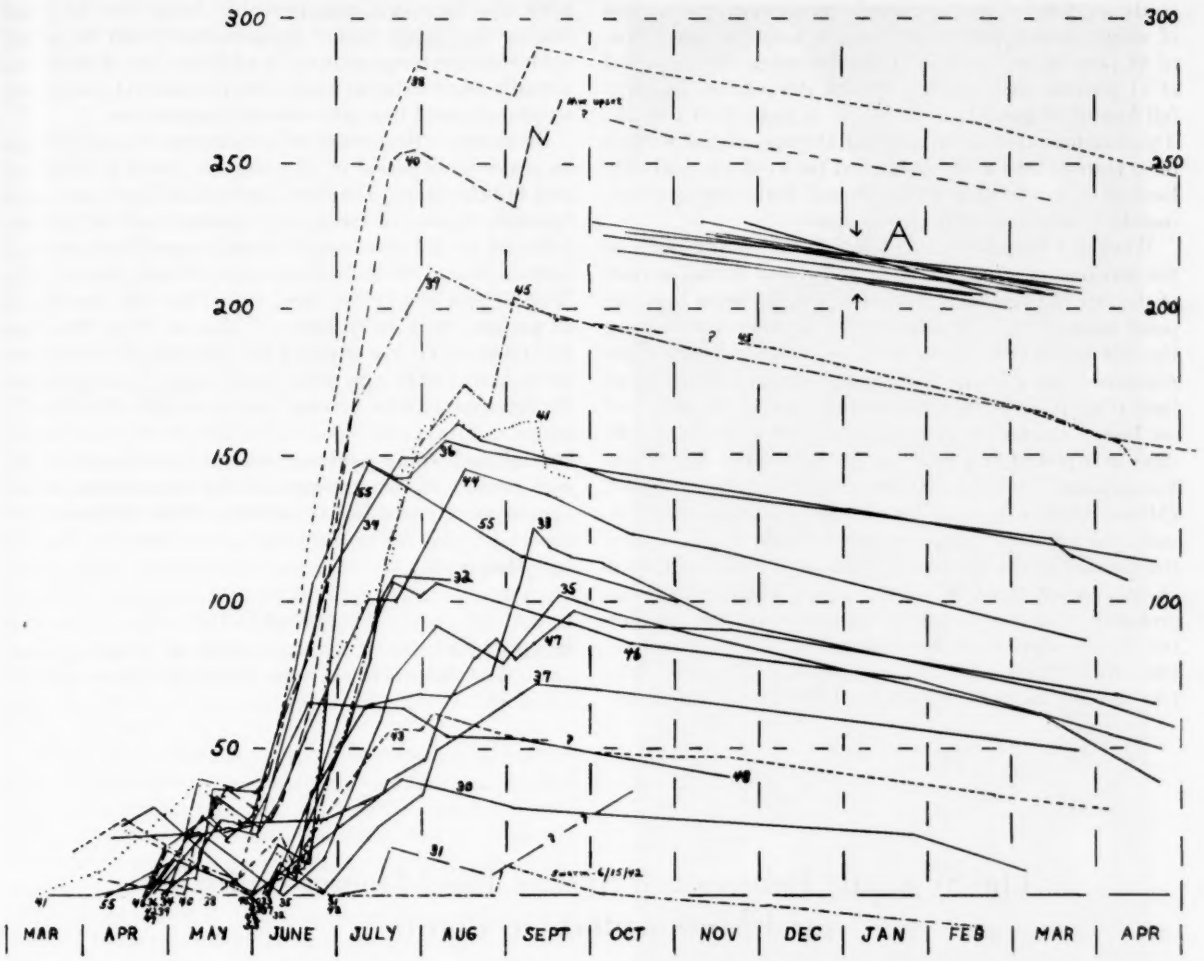

FIG. 1.—Weight changes of a honey bee colony over a period of 20 years, 1930–1948, and 1950, as listed in table 1. The lowest weight of each year (spring or summer) is reduced to zero and recorded on a common base line, except 1942. The upper peak of each line is the cumulative total of the respective yearly weight gain. The group of lines at *A* represents the corresponding period of decline for all years grouped for purposes of comparison.

to best provide for them in the matter of furnishing stores or room for surplus storage. The variation in loss of 24 to 63 pounds from the high point of the fall (August 4-September 24) to the low of the spring (March 14-May 6), with an even greater actual consumption of stores, gives beekeepers a valid reason for killing of colony populations at the close of the honeyflow or even dequeening at an earlier date. However, under such management the producer must be sure that he can obtain package bees at an optimum spring date and so manage them that the net returns are at least equal to that from overwintered colonies.

The area of Champaign-Urbana, Illinois has a variable spring honeyflow as indicated by increases in weight of 2 to 27 pounds ranging between April 19 and May 22, the nectar source being dandelion and fruit bloom. During the following dearth period varying from May 15 to June 11 over the 20-year period, the losses in weight varied from 2 to 32 pounds. Since this is an important build-up period in brood rearing and consequent colony population, the producer should check all colonies to see that stores have not been depleted to the point of starvation or retardation of brood rearing.

For the main summer flow, the weight gains varied from 14 to 284 pounds from June 10 to August 11. The low gains for this period of 14, 15, and 16 pounds were of a colony found queenless of June 4 of a good honey year, possibly after swarming, and the other two in years of practically no surplus honeyflow. The 5 years of largest gains (160, 160, 178, 230, 284 lbs.) resulted from summer honeyflows extending well beyond the usual blooming period of sweet clover.

For the fall period, with the exception of 2 years, one with a small gain and the other with incomplete records, the decrease in weight varied from 2 to 22 pounds between July 22 and September 3. No doubt, a part of the stores from these fall flows was replaced by the weight of brood developed as a result of the honeyflow stimulus. For 15 of the 18 years with complete records, there was a fall gain of 3 to 60 pounds with the peak varying from August 4 to September 24.

If the gains and losses of the years with complete re-

cords are totaled and averaged, an average winter loss of weight is indicated as 40 pounds, a spring honey flow of 21 pounds, with a loss in the following dearth period of 11 pounds, then a honey flow of 105 pounds, an early fall loss of 10 pounds, followed by a gain of 20 pounds. The summation of these gives an average annual surplus of 85 pounds over a 20-year period for a colony, centrally located in a city area whose closest limits are approximately 1 mile distant from the apiary.

WINTER CONSUMPTION.—Another interesting point is the amount of stores actually consumed during a part of the late fall and early winter period. In figure 1, at the point marked "A", the portions of the lines representing the fall and winter losses over the extended period are reproduced as a group. Laying a rule over this group of lines it is apparent that the average loss in the period of no brood rearing is approximately 20 pounds in 140 days or 1 pound in 7 days, or 2.3 ounces per day. Even this amount is in excess of that given in a previous report (Milum 1929) where the lowest loss of 10 unpacked colonies was 1.08 ounces per day and the highest 1.97 ounces, the average of the 10 colonies being 1.41 ounces during a 76-day period from November 15 to January 30, this probably being a period of little or no brood rearing. For the first 97 days from November 15, 1927 to March 2, 1928, the average loss in weight per day per colony was 1.82 ounces, and 2.09 ounces for 123 days to March 18, 1929, the increased rate probably being due to brood rearing (requiring higher temperatures) and to actual colder outside temperatures. In addition, loss of stores was actually offset so far as weight was concerned by increased weight of young bees added to the populations.

SUMMARY.—Records of weight changes of a colony kept on scales for a period of 20 years are shown in graphical and tabular form. The lows (pounds of loss) and highs (pounds of gain) of the spring, summer and fall periods, followed by the extremes of dates, respectively, were as follows: loss of 24 to 63 (from high of fall), March 14 to May 6; gain of 2 to 39, April 19 to May 22; loss of 2 to 32 pounds, May 15 to June 17; gain of 14 to 284, June 10 to August 11; low of plus 2 to −22, July 22 to September 9; loss of 10 to gain of 60, from August 4 to September 24. An approximate average loss in weight of stores of 1 pound in 7 days or 2.3 ounces per day is indicated for the late fall and winter nonbrood rearing period over the 20-year period; this comparing with the average loss of 1.41 ounces per day of a 76-day period in 1928–1929, and 2.09 ounces per day for the 123-day period between Nov. 15 and March 18.

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The Systemic Behavior of Systox Thiol Isomer Sulfoxide and Methosulfate in Plants[1,2]

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Two derivatives of the thiol isomer of Systox[4] (*O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate) namely, its dimethylsulfonium salt (hereafter designated as the thiol isomer methosulfate); and *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (hereafter designated as the thiol isomer sulfoxide), have been described as effective systemic insecticides (Fukuto *et al.* 1955a, 1955b). The former compound is a completely water-miscible derivative of the thiol isomer, and the latter has been isolated and characterized as a major toxic metabolite of the thiol isomer in plant tissues (Fukuto *et al.* 1955a, Fukuto *et al.* 1956). This paper evaluates the comparative systemic behavior of these two compounds as compared with the thiol and thiono isomers of Systox, as studied by means of P^{32} radiotracers.

MATERIALS AND METHODS.—The thiol isomer sulfoxide was prepared by the oxidation of the thiol isomer in aqueous H_2O_2 (Fukuto *et al.* 1955a). The purified compound was 99.9+% homogenous in paper chromatography upon propylene-glycol impregnated paper (Fukuto *et al.* 1955a) and had a relative activity of 12.74 counts per second per microgram on September 29, 1955. The thiol isomer methosulfate was prepared as described by Fukuto *et al.* (1955b) and had a relative activity of 9.85 c.p.s. per microgram on 9/29/55.

The technics used for radiotracer evaluation and handling have previously been described (Metcalf *et al.* 1954, Fukuto *et al.* 1955a).

DISCUSSION OF RESULTS.—*Relative Rates of Absorption and Translocation by Plants.*—A comparison was made of the relative rates of absorption and translocation of the thiol isomer and its sulfoxide and methosulfate by topically treating the basal stems of young cotton plants with equimolar amounts. The quantities used were: thiol isomer, 10λ(11.3 mg.); thiol isomer sulfoxide, 10λ(12 mg.); and thiol isomer methosulfate 11.6λ(15.4 mg.). Three plants were treated with each material and the amount of material absorbed and translocated to the growing leaves was measured by combining equal-sized

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[4] Trade-mark, Chemagro Corporation, New York, N. Y.

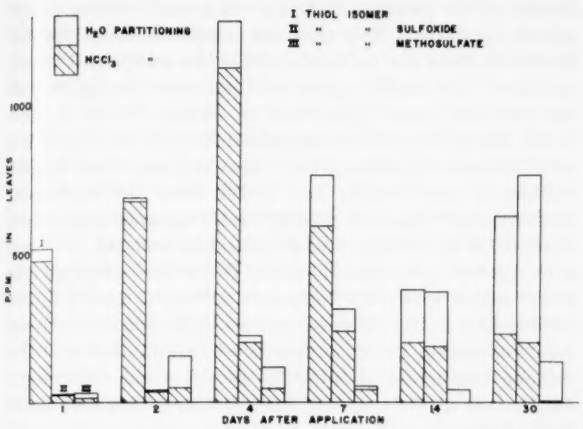

FIG. 1.—Accumulation of P^{32}-labeled Systox thiol isomer, thiol isomer sulfoxide, and thiol isomer methosulfate in leaves of cotton plants at various intervals after application to stem.

pieces of the leaves from each replicate (aggregating 0.3 gm.) homogenizing in 10 times their weight of water and evaporating aliquots in planchets for assay. The results are represented in figure 1, and show the total amounts of radioactivity in the leaves at intervals after treatment, and the $HCCl_3/H_2O$ partition coefficients of the radioactive material.

It is clear that the thiol isomer was absorbed and translocated much more rapidly than the sulfoxide, up to 7 days after application. At 14 days the amounts were nearly equal, and at 30 days the sulfoxide was present in appreciably greater amounts. The concentration of the methosulfate built up most slowly and increased from about 4% of that of the thiol isomer at 1 day to 16% after 30 days. From this information and from the performance data on these systemic materials (Fukuto *et al.* 1955a, 1955b), it appears that the sulfoxide provides a considerably longer-lasting systemic effect than the thiol isomer and that the methosulfate may persist even longer. The sulfoxide is more polar and less lipoid-soluble than the thiol isomer, and the methosulfate, which is water-miscible, is strongly polar and of low lipoid solubility. It appears therefore that the degree of lipoid solubility strongly influences the rate of absorption through the waxy plant cuticle.

More information on the relative rates of absorption is given by the radioautographs in figure 2. Tiny droplets of approximately 0.1λ of the systemics were placed in the center of young lemon leaves, and radioautographs were made at intervals of 1, 2, 4, 7, and 14 days. The 14-day autographs are typical and show the immobility of the methosulfate, the small degree of penetration of the thiono isomer and of the thiol isomer sulfoxide, and the very effective dispersion of the thiol isomer. This comparative behavior is similar to that of *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiolate and its oxalate (to be described elsewhere), the free base penetrating far more effectively than the salt, which, however, is a very active long-lasting systemic.

It is apparent from these factors that the length of duration of systemic activity depends in part upon the rate of absorption of the compound into the plant juices,

FIG. 2.—Radioautographs showing dispersal of P^{32}-labeled systemics 14 days after application as 0.1-microliter droplets at the center of young lemon leaves: Systox thiol isomer (upper left), Systox thiono isomer (lower left), thiol isomer sulfoxide (upper right), thiol isomer methosulfate (lower right).

where it is exposed to destructive aqueous hydrolysis and enzymatic action.

Plant Metabolism.—The biochemical behavior of the P^{32}-labeled Systox thiol isomer and its sulfoxide and methosulfate in young cotton plants was studied by means of paper chromatography on propylene-glycol-impregnated paper, as previously described by Fukuto *et al.* (1955a). Chromatograms of the chloroform-soluble fractions of the leaf homogenates were made at intervals of 1, 2, 4, 7, 14, and 30 days, and the material in the various spots was quantitatively determined by measurement of radioactivity.

The data obtained with the thiol isomer and its sulfoxide are plotted in figure 3. The information is good agreement with the previous study of the metabolism of the thiol isomer in plants (Fukuto *et al.* 1955a). Only

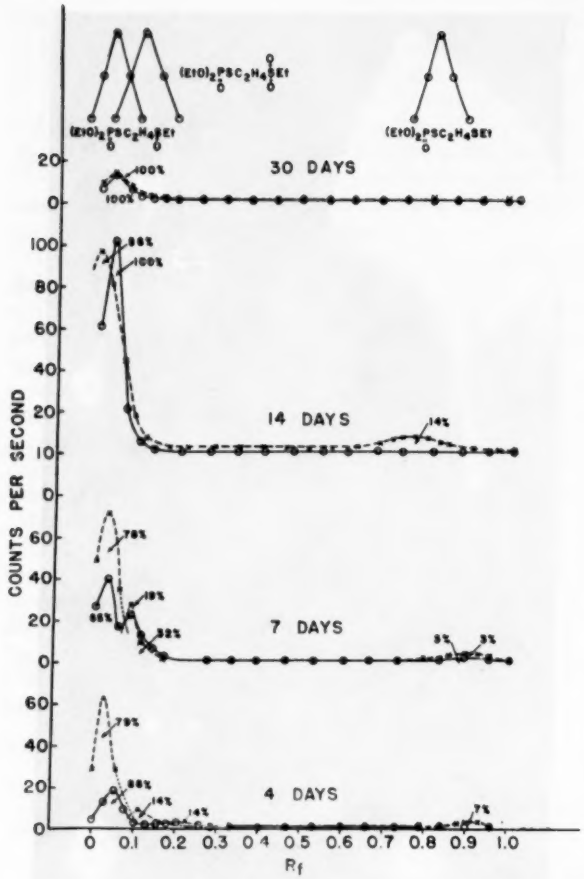

FIG. 3.—Propylene glycol paper chromatograms of chloroform extracts of leaves of cotton plants at various intervals after topical application of P^{32}-labeled Systox thiol isomer and its sulfoxide.

traces of the thiol isomer (R_f 0.8–0.9) were found in the leaves from thiol-isomer-treated plants at 4, 7, and 14 days. This indicates its rapid metabolism to a compound (R_f 0.00–0.04) which has been identified as the sulfoxide by its paper-chromatographic behavior, its biological properties and by its isolation and infrared characterization (Fukuto *et al.* 1955a, 1956).

The sulfoxide is quite evidently the principal toxic plant metabolite of the thiol isomer and, as shown in figure 2, is the principal metabolite present in the chloroform-soluble fraction of thiol isomer-treated cotton leaves for at least 30 days after treatment. From a study of the chromatographic profiles in figure 3 and, especially, of the original radioautographs of these chromatograms, it is evident that a secondary metabolite is also present with a slightly higher R_f (0.03–0.08). This compound has been identified with certainty as the thiol isomer sulfone *O,O*-diethyl *S*-2-ethylsulfonylethyl phosphorothiolate by its chromatographic behavior as studied by radiotracers, its biological and chemical properties, its isolation from cotton plants, and by its infrared characterization (Fukuto *et al.* 1956).

The R_f of the thiol isomer sulfone was originally described as O from colorimetric determinations. However, the use of the radiotracer has given a more precise determination, and it is clear that this compound has a slightly higher R_f than the sulfoxide and is the compound which produced the smaller upper spot described in figure 4 of the previous paper (Fukuto *et al.* 1955a). In the 4- and 7-day leaves this sulfone comprised from 14 to 19% of the total chloroform extractives. However, the thiol isomer sulfone is considerably less stable than the sulfoxide, hydrolyzing 6 times as rapidly under aqueous conditions (Fukuto *et al.* 1955a), and its presence was not detected after periods of 14 and 30 days. When the cotton plants were treated with the thiol isomer sulfoxide, a more direct metabolism to the sulfone occurred, since the preliminary plant oxidation to the sulfoxide was eliminated and the sulfone comprised about 32% of the total chloroform extractives at 7 days, but again was not detected at 14 and 30 days.

The metabolism of the thiol isomer methosulfate is more difficult to elucidate because of the relatively small quantities present in the plant tissue. The methosulfate has an R_f of O on the propylene glycol system, and the radioautographs of the chloroform extractives show faint spots at this location in 7-, 14- and 30-day leaves. The 7- and 14-day leaves also show a distinct second spot, with a slightly higher R_f, at the same position as that of the thiol isomer sulfone. However, from the electronic structure of the thiol isomer methosulfate it seems improbable that oxidation to compounds such as the thiol isomer sulfoxide or sulfone can occur. The identity of this compound must await further investigation.

The data on partitioning of the radioactivity in the treated cotton plants between chloroform and water (fig. 1) are indicative of the hydrolytic decomposition of the chloroform-soluble thiol isomer and thiol isomer sulfoxide, which partition 98% or more into the chloroform phase, to the predominantly water-soluble diethyl phosphoric acids, which partition about 98% in the water phase. Figure 1 shows that the rates of decomposition of the radioactive material in plants treated with thiol isomer and thiol isomer sulfoxide are comparable, as is to be expected, since the former is rapidly converted to the latter *in vitro*. The biological half life in cotton leaves of the thiol isomer sulfoxide was evidently slightly more than 14 days, in very good agreement with the value of 18 days previously determined for the thiol isomer (Metcalf *et al.* 1955).

The thiol isomer methosulfate has a chloroform/water partition ratio of 0.055 and is therefore almost entirely in the water phase. The data presented in figure 1 show that cotton plants treated with this material contain P^{32} activity which partitions mostly in the water phase. This suggests that there is little transformation to predominantly chloroform-soluble metabolites. However, the hydrolytic decomposition products of this compound also partition in the water phase, so that the data give no information on the rate of degradation of the compound. The increased electron-withdrawing properties of the dimethylsulfonium group undoubtedly decrease the hydrolytic stability of the thiol isomer methosulfate which should shorten the residual life of the material actually absorbed into the plant.

SUMMARY AND CONCLUSIONS.—The systemic behavior of the Systox thiol isomer and its sulfoxide and metho-

sulfate derivatives when applied topically to stems of cotton plants has been studied by means of P^{32} radiotracers and paper chromatography. The accumulation of radioactive materials in the leaves of these plants was most rapid with the thiol isomer up to a period of 14 days after application; after that period the sulfoxide treatment gave the higher values. The much slower accumulation of radioactivity from the methosulfate indicated a lower degree of penetration of this polar compound through the plant cuticle. Radioautography after topical application of these systemics to young lemon leaves showed that the order of penetration and spread in the leaf interior was thiol isomer methosulfate < thiol isomer sulfoxide < thiono isomer < thiol isomer. The rates of metabolism and decomposition of the thiol isomer and sulfoxide were approximately the same, and small amounts of thiol isomer sulfone formed from both treatments.

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Multiple Mating of Queen Honey Bees[1]

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For many years it was generally believed that queen honey bees, *Apis mellifera* L., mate only once during their life time. However, studies by various investigators in recent years, including those by Triasko (1951) and Taber (1954) have indicated that queens may mate several times prior to initial egg laying.

Triasko dissected queens which had just returned from mating flights, and from a comparison of the volume of sperm in their oviducts with that in the drone's seminal vesicles he estimated that each queen mated with four or five drones. Taber allowed mutant queens to mate in an area stocked with uncontrolled populations of mutant and wild-type drones. The queens were classified, according to their offspring as follows: (1) offspring of both types, (2) offspring all wild-type, and (3) offspring all mutant. The class frequencies were compared with a theoretical frequency table derived by substituting various population proportions of mutant and wild-type drones and different numbers of matings into the binomial $(p+q)^n = 1$, where

p = the probability of mating with a mutant drone,
q = the probability of mating with a wild-type drone, and
n = the number of matings per queen.

Taber stated that the queens mated " . . . on an average $6\frac{1}{2}$ times."

In an attempt to obtain further information on the extent of multiple mating, tests were conducted at Algonquin Park and Ottawa, Ontario, during the summer of 1955. The experimental and analytical procedures in these tests were similar to those used by Taber; however, in the Algonquin tests the proportion of mutant and wild-type drones was controlled, thus eliminating one of the analytical unknowns. The results of the work are presented in this paper.

METHODS.—The simple recessive mutation, cordovan, discovered at the Beltsville Research Center, which produces brown or cordovan body pigment in place of black (Mackensen 1951), was used in this study as a genetic marker. There is no evidence to indicate that this mutation is in any way harmful.

Hybrid cordovan virgin queens were reared using a double graft technique. They were unrelated to the cordovan drones in the two tests apiaries, and thus would be expected to carry different lethal alleles (Mackensen 1951). High viability was expected and obtained in brood from all matings of these queens and drones.

An experimental apiary site was established at Canoe Lake in Algonquin Park because there were no bees located within 40 miles, according to the provincial colony registration records. To ascertain that this area contained only experimental bees, a mating purity test was conducted. Ten colonies headed by cordovan queens and thus stocked with only cordovan drones were moved to the site. Also located at the site were 50 droneless 5-frame nuclei stocked with cordovan virgins. Forty-two of these queens mated successfully and produced only cordovan offspring. Had there been other bees in the area, some of the cordovan virgins would have mated with wild-type drones and consequently would have produced at least some wild-type offspring.

The 10 cordovan colonies were removed from the area and replaced by eight others headed by heterozygous cordovan queens. To test whether such queens produce a 1:1 ratio of cordovan and wild-type drones, all the drones from another eight colonies headed by heterozygous cordovan queens were killed and the numbers of cordovan and wild-type counted. There was a total of 3,827 cor-

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dovan drones and 3,983 wild-type drones ($X^2=3.116$). It may be assumed then that the drone population of the Algonquin colonies was one-half mutant and one-half wild-type.

Throughout the season a total of 164 cordovan queens were mated to the 1:1 drone population. The queens were grouped according to their offspring into the three classes: (1) offspring of both types, (2) offspring all wild-type, and (3) offspring all mutant. Those in class (1) mated to one or more cordovan drones and one or more wild-type drones: those in class (2) mated with one or more wild-type drones; and those in class (3) mated with one or more cordovan drones.

The groupings of the 164 queens into the three classes are as follows:

Number of Queens	Progeny	Relative Frequency
162	Wild-type and mutant	0.9878
1	Wild-type only	.0061
1	Mutant only	.0061

With the known drone population one-half mutant and one-half wild-type and assuming that 164 queens mate an average of seven times, the following expectations are derived:

Number of Queens	Progeny	Expected Frequency
161.44	Wild-type and mutant	0.9844
1.28	Wild-type only	.0078
1.28	Mutant only	.0078

The relative frequencies calculated from the observed results are so similar to the expected frequencies where seven matings are substitutied that it may be considered the Algonquin queens mated approximately seven times on the average.

Related tests were conducted at Ottawa where 56 colonies headed by cordovan queens produced only cordovan drones and eight colonies headed by wild-type queens produced only wild-type drones. This apiary was located in an area where there were an unknown number of other colonies; thus the wild-type drone population was unknown. It can be safely assumed that the unknown drone population was all wild-type since the first cordovan stock was introduced to the area in the fall of 1954.

Throughout the season a total of 303 cordovan queens were mated from 5-frame nuclei located in this apiary. The queens were grouped according to their offspring into the three classes as follows:

Number and Queens	Progeny	Relative Frequency
293	Wild-type and mutant	0.9670
9	Wild-type only	.0297
1	Mutant only	.0033

By substituting a drone population two-fifths mutant, three-fifths wild-type and 303 queens mating an average of seven times, the following expectations are derived:

Number of Queens	Progeny	Expected Frequency
294.03	Wild-type and mutant	0.9704
8.48	Wild-type only	.0280
.49	Mutant only	.0016

Again the relative frequencies are so similar to the expected frequencies, where seven matings and a two-fifths mutant and three-fifths wild-type drone population are substituted, that it may be considered the Ottawa queens also mated approximately seven times on the average.

DISCUSSION.—The purpose of this study was to obtain information on the number of times queen honey bees mate.

The Algonquin Park site was chosen because it was isolated and thus the experimental drones could be controlled. Wherever the mutant queens mated, the cordovan to wild-type drone population ratio was 1:1. The Ottawa site was chosen because it was similar to the one described by Taber. The results therefore would be comparable.

If the cordovan mutation is harmful to the mating behaviour of the drone, then where the ratio of cordovan to wild-type is 1:1, the number of queens mated to only wild-type drones would be significantly greater than the number mated to only cordovan drones irrespective of how many times queens mate. On the other hand, if the mutation is beneficial the reverse would be true. Since in the Algonquin tests the number of queens mated to only cordovan drones was the same as the number mated to only wild-type drones, it may be assumed that the mutation does not affect mating behaviour.

Data from the 164 queens mated in Algonquin Park and the 303 queens mated at Ottawa suggest that the queens of both groups mated, on the average, approximately seven times. This figure agrees essentially with the average of $6\frac{1}{2}$ matings calculated by Taber. It is somewhat greater than the four or five matings estimated by Triasko; however, his estimate is based on only one mating flight whereas many queens mate on two or more flights (Oertel 1940, Roberts 1944, Alber *et al.* 1954, Ruttner 1954).

The use of the binomial presupposes that all queens mate the same number of times. It is more logical to assume that there is a considerable range between the minimum and maximum number of matings. Probably, individual queens have different sperm-storage capacities. Certainly, a different amount of sperm is received from each drone—this method of analysis also assumes that the amount of sperm received from each drone is the same. At present it is not feasible to determine the range of matings because it is not possible to observe the mating act, nor is it possible to phenotypically segregate the offspring of individual queens to ascertain how many times they have mated. Nevertheless, the binomial should give a reasonably close average of the number of times queens mate.

In all probability several suitable mutant markers will be available in the future. It will then be possible to study the entire problem of multiple mating in more detail.

SUMMARY.—Statistical analysis of offspring-type data from 467 genetically-marked queens, mated in areas containing both wild-type and genetically-marked drones, indicate that the queens mated an average of seven times.

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The Behavior of Nosema-Infected Bees Affecting Their Position in the Winter Cluster[1]

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Nosema disease is responsible for heavy losses to the beekeeping industry, since it causes queen supersedure and retards the development of package bees and overwintered colonies. The Nosema parasite does not kill the bees outright or cause them to manifest specific symptoms. However, it shortens their life up to 50%, and queens are superseded within 2 to 6 weeks after becoming infected. Thus, Nosema is more a disease of the colony than of individual bees. Because Nosema affects colony strength, it is essential either to control the disease or to assay its intensity in colonies used in research on management, breeding, or bee behavior. Reliable assay methods are equally essential for evaluating control measures.

At the Beekeeping Laboratory at Madison, Wisconsin, overwintering colonies are sampled at monthly intervals. Samples are taken from the top center of the clusters. To determine the presence of the parasites, 10 bees are macerated in a small amount of water and examined under a microscope. If they are positive, individual ventriculi from 10 additional bees are examined to obtain an estimate of the level of infection within the population.

This paper deals with an investigation on the behavior of Nosema-infected bees within the winter cluster as it may affect sampling methods for estimating infection in overwintering colonies. Tests were made in 1953 and 1955 to determine whether there is a tendency for localization of infected bees in the winter cluster. This information will aid the inspector in choosing the proper area for sampling colonies.

PROCEDURE.—An overwintering colony was killed with hydrocyanic acid gas when the bees were tightly clustered at low temperature. The fumigant was introduced into the hive through an auger hole provided with a rubber tube connected to the generating flask. The gas killed the bees before they had an opportunity to alter their position. The hive was then placed on its side, and the combs were lifted out horizontally to prevent the bees from shifting their position in the cluster. Samples of bees for each interspace were then taken from 1-square-inch areas marked off with a wire grid placed over the upper face of each comb. The bees were kept in a frozen condition until the sampling was completed. To determine the presence of the parasites, individual bees were macerated in a small amount of water and examined under a microscope. The oval, highly refractile Nosema spores were readily recognized at a magnification of 40 diameters, but 440× was generally used.

COLONY TESTS.—The first attempt to determine distribution of infected bees was made by C. L. Farrar on a colony killed on January 22, 1943. The colony was of normal size with three frames containing brood. Two bees were examined from each square inch of comb covered by the cluster. Out of 1573 bees examined, 3.7% were infected. Thirty per cent of the infected bees were found on the brood areas, 30% within the cluster center but off the brood areas, 33% at the top, and only 7% within the 1- to 2-inch insulating surface band of bees below the top. The author has continued this study with two other colonies.

A colony killed on February 3, 1953, covered portions of seven standard Langstroth combs. The percentages of infected bees occupying the interspaces between these combs are shown in table 1. The comb between interspaces 5 and 6 contained 5-×7-inch areas of brood on both sides, and each of the adjacent combs had smaller areas of brood on one side facing these interspaces. Samples were taken from square-inch areas distributed uniformly over the combs. Ten bees were used, except in peripheral areas where there were less than 10 bees within the square inch.

Of particular interest is interspace 5, in which 29 of a total of 85 square inches were adjacent to brood. The position of infected bees in relation to this brood area is shown in figure 1. The presence of emerging bees reduced the percentage of infected bees in the brood area. For example, if half of a 10-bee sample were young bees, the percentage of infected bees would be reduced by half. Infected bees in the other interspaces were concentrated in the upper portions of the cluster. It appears that infected bees do tend to concentrate in and immediately above the brood areas, which represent the warmest regions of the cluster.

A different technique was used in 1955 to eliminate the

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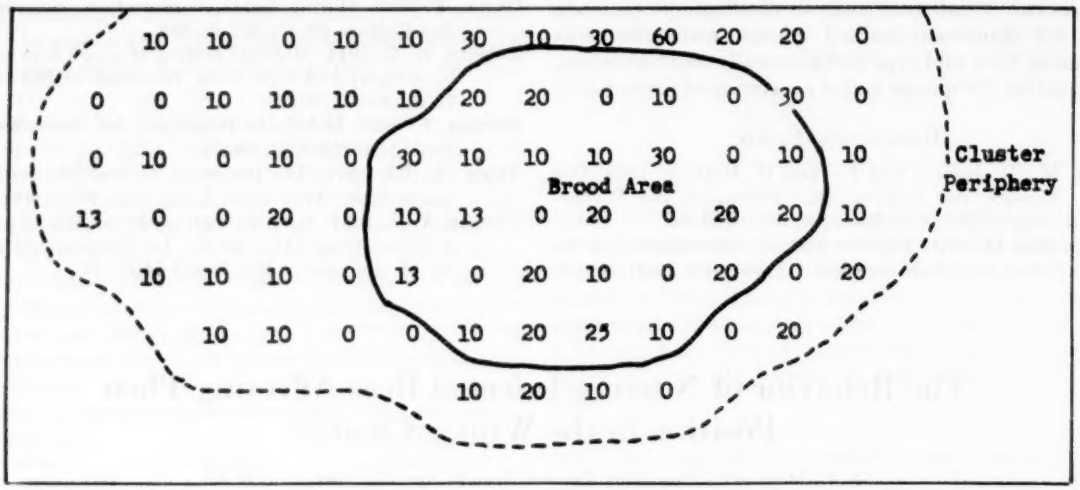

FIG. 1.—Distribution of Nosema-infected bees in interspace 5 of a colony killed on Feb. 3, 1955. Numbers show the percentage of infected bees in the respective square-inch areas.

need for individual diagnoses of large numbers of bees. On January 19, 500 bees were caged in the laboratory, marked with yellow paint on the thorax, and fed a suspension of Nosema spores in sugar sirup. Four days later these inoculated bees were introduced to a colony, together with 300 Nosema-free check bees marked red. This colony had been fed fumagillin in the fall to prevent Nosema disease. Three weeks later the colony was killed and the positions of the two groups were determined as before. Samples of the check bees showed no infection, and a sample of the inoculated bees showed infection in 5 to 7 days after inoculation.

When killed on February 8, the colony had two combs of brood and occupied nine interspaces in shallow hive equipment (frames $6\frac{1}{4}$ inches deep), extending from the inner cover through one hive body and $1\frac{1}{2}$ inches down into the body beneath. A total of 59 inoculated and 128 check bees were recovered. Apparently the 20-day interval from the time of inoculation allowed a heavy mortality; thus, fewer bees were found than desirable. A 10-day interval would have been preferred, but it was necessary to wait for a temperature low enough to insure a tight cluster. However, a distinct pattern of distribution was obtained from the small number surviving, as shown in table 2.

Table 1.—Distribution of Nosema-infected bees in a colony killed on Feb. 3, 1953.

Interspace	Square Inches of Comb Occupied	Number of Bees Sampled	Per Cent of Bees Infected
1	2	10	0
2	19	75	12
3	47	123	10
4	70	200	19
5	85	752	11
6	78	720	9
7	32	153	14
Top of cluster		48	38
Total or average		2081	11.7

It will be noted that the survival of check bees was three and one-half times that of the inoculated bees. Nearly twice as many inoculated bees were found per square inch in and above the brood area as in the broodless area and four times the number in the top half of the cluster as in the bottom half.

In addition to the test bees introduced, 940 unmarked bees from various portions of the cluster were found to be Nosema-free. Most of these bees (830) were from the brood-area interspaces. Apparently the colony was Nosema-free except for the badly infected bees that were introduced.

DISCUSSION.—The tendency of Nosema-diseased bees to congregate in the warmer parts of the winter cluster permits taking samples from the top bars at the center of the cluster with an excellent chance of obtaining infected bees if any are present. They can be taken quickly and with a minimum of disturbance to the colony by scooping the bees into a salve box. Ten-bee samples probably will permit detection of infection greater than about 10% but may not disclose lighter infections.

Colonies that have participated recently in a winter cleansing flight will show less infection than those confined for a period of weeks. Following a flight, bees may be found on the surface of the snow within a radius of a quarter of a mile from the apiary. Samples collected from

Table 2.—Distribution of marked inoculated and check bees[a] in a colony 3 weeks after their introduction, 1955.

Portion of Cluster	Number of Bees		Per Cent of Bees Introduced		Infected Bees per Square Inch
	Infected	Check	Infected	Check	
In and above brood area	23	29	5	10	0.109
Broodless area[b]	36	99	7	33	.063
Top half	49	71	10	24	.115
Bottom half	10	57	2	19	.028
Entire cluster	59	128	12	43	.075

[a] When examined microscopically, all inoculated bees were heavily infected, and all check bees were Nosema-free.

[b] All areas of the cluster except where brood was present and immediately above it.

the snow usually show several times the percentage of infection exhibited by the average for all colonies in the apiary.

Infected bees not only seek the warmer regions of the cluster, but they are also stimulated to fly by the distress caused by the Nosema parasite. Winter flights are beneficial even though a considerable number of bees become chilled and fail to return to the hive. The gain resulting from the elimination of infected bees is usually greater than the loss due to a reduction in total population.

Biology of *Bdella depressa*, a Predaceous Mite[1]

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Incidental to a winter study of the clover mite, *Bryobia praetiosa* Koch, in Champaign-Urbana Illinois, extraordinary numbers of reddish-yellow, spiny mite eggs reminiscent of minature naval mines or the ball-like fruit of the sweetgum tree were collected from the basal bark of sycamore, elm, maple, and oak trees. Sometimes five or more sharp-snouted mites and 900 or more of these eggs were counted per 100-gram sample of bark. These "snout mites" and the spiny eggs belonged to the species *Bdella depressa* Ewing of the family Bdellidae, order Acarina. Because of the abundance of this mite in bluegrass waste areas, in orchard cover, and in lawns near trees, where clover mites are sometimes a problem, this bdellid was studied in the field and reared in the laboratory during 1955 to determine if it was a predator of the clover mite.

In early April the eggs of this bdellid hatched and the small, voracious larvae diligently searched the bark and soil debris for mite eggs, active mites, and springtails. The larvae, nymphs, and adults were observed to feed on all stages of the clover mite; the two-spotted spider mite, *Tetranychus telarius* (L.); the brown wheat mite, *Petrobia latens* (Müll.); two additional species of mites, *Petrobia harti* (Ewing) and *Schizotetranychus* sp.; and two species of Collembola, the garden springtail, *Bourletiella hortensis* (Fitch), and *Willowsia platani* (Nicolet). This species of bdellid spears its prey with its sharp chelicerae, then hangs on and feeds until the prey is subdued and finally sucked dry.

During the spring, very late summer, and early fall, *B. depressa* is abundant in most grassy areas in the vicinity of Champaign-Urbana. This mite seems to prefer cool weather and moist conditions. With the approach of cold weather in October great numbers of adults were observed under the basal bark of trees in grassy areas. At this time the female deposits eggs in tight, dry crevices and fissures on the innerside of basal bark. In addition to hibernating under tree bark, this species to some extent hibernates in grassy debris. Although the egg stage is the most abundant overwintering stage, all stages, except the larval stage, were observed to overwinter.

Mites were reared in the laboratory in pint screw-top fruit jars. Each jar contained about 150 ml. of a humidity-regulating salt solution. Sets of three jars with salt solutions producing relative humidities of approximately 28%, 75%, and 90% were used. One set of the three salt solution jars was placed at each temperature in constant temperature cabinets held at 39° F., 47° F., 60° F., 70° F., 85° F., and 90° F. A glass dish 2 inches in diameter and 2 inches in depth was placed in each jar to serve as a container for bark infested with *B. depressa* eggs. A ring of petrolatum was applied around the top of each dish to restrain the mites when they hatched.

On February 15, 1955, pieces of bark infested with the eggs of this species of bdellid were collected in the field and brought to the laboratory. Here 18 samples were sorted so as to have a known number of eggs varying between 50 and 100 per sample. One sample was placed in each dish and the number of eggs in each dish was recorded. Daily observations were made on egg development and hatching. Since the eggs had been exposed to the natural environment during part of the fall and winter, some development may have taken place in the eggs previous to collection. Consequently, the incubation periods given in table 1 may actually be shorter than those occurring in nature.

After the larvae emerged from the eggs they were kept under the same temperature and humidity conditions as the eggs. A known number of clover mites or two-spotted spider mites on rose or apple leaves was placed in the open-top dishes as food for *B. depressa*. Food changes, observations on prey consumed, rate of development, and losses of mites in the petrolatum barrier were made each day. Specimens of each stage of development were preserved.

In the development of *B. depressa* the egg, the deutovum, the larval, the protonymphal, the deutonymphal, the tritonymphal, and adult stages occur in the female and presumably in the male. The termination of the larval and nymphal stages end in quiescent periods. It is apparent from table 1 and from observations that the duration of the stages is influenced markedly by temperature and in the active stages by the success in obtaining food. At 90% relative humidity and temperatures of 60° F. and 70° F., where rearing was successful, this species fed quite readily on the clover mite and the two-spotted spider mite. Three or more mite eggs or two or more larval prey mites were required to complete the larval development of *B. depressa*. To reach adulthood each nymphal bdellid of this species required two, three, or more nymphal or adult prey mites to complete each stage. Development from larva to adult varied from 21 to 30

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Table 1.—Development of *Bdella depressa* on clover mites and two-spotted spider mites under regulated temperatures and humidities.

Degrees F.	Per Cent R.H.	Per Cent of Eggs Developing into Deutovums	Per Cent Hatch	Approximate Duration of Stages (Days)						
				Egg[a]	Deutovum	Larva	Deuto-nymph	Proto-nymph	Trito-nymph	Larva to Adult
90	90	0	0	—[b]	—	—	—	—	—	—
90	75	0	0	—	—	—	—	—	—	—
90	28	0	0	—	—	—	—	—	—	
85	90	96	87	6	<1	1–2	2–3	—	—	—
85	75	3	0	—	—	—	—	—	—	—
85	28	0	0	—	—	—	—	—	—	—
70	90	96	94	8	1	1–2	2–3	5–8	6–8	14–21
70	75	94	94	10	1	1–2	2–3	—	—	—
70	28	2	0	—	—	—	—	—	—	—
60	90	90	89	22	6	1–4	4–6	8–10	8–10	21–30
60	75	83	67	24	6	1–4	4–6	—	—	—
60	28	0	0	—	—	—	—	—	—	—
47	90	31	13	42	9–12	—	—	—	—	—
47	75	15	8	40	9–12	—	—	—	—	—
47	28	0	0	—	—	—	—	—	—	—
39	90	0	0	—	—	—	—	—	—	—
39	75	0	0	—	—	—	—	—	—	—
39	28	0	0	—	—	—	—	—	—	—

[a] Eggs were collected in the field February 15, 1955.
[b] — indicates no development.

days at 60° F. and 90% relative humidity, and from 14 to 21 days at 70° F. and 90% R.H. (table 1). Some degree of development was obtained at temperatures and humidities close to these two successful ones, but no development was observed at 90° F. and 39° F. With the exception of two eggs at 70° F. no development was observed at 28% R.H.

Apparently in grassy areas *B. depressa* is an important predator of the clover mite. In some areas studied 75% or more of the clover mite predators were of this species of bdellid. There are certain similarities in the life cycles of these two species which seem to contribute to this situation: (1) both species are only abundant in spring and fall, apparently due to summer diapauses; (2) both species overwinter primarily as eggs in the same types of habitats; (3) the dates of hatching of the overwintering eggs are very close to each other; and (4) both species require humid and cool conditions. On the other hand, this bdellid seems not to be so important as a predator of the two-spotted spider mite. This apparently is because conditions favorable to the two-spotted spider mite are unfavorable for this bdellid. However, it is possible that it may feed upon the overwintering two-spotted spider mite to a considerable extent during early spring and late fall. The role *B. depressa* plays in controlling other mites and springtails is unknown.

SUMMARY.—High populations of a bdellid mite, *Bdella depressa* Ewing, were observed overwintering during the spring and fall of 1955 under the bark of trees and in grassy areas in the vicinity of Champaign-Urbana, Illinois. This mite was a predator on the clover mite, other species of mites, and Collembola. In the course of its development the bdellid mite passed through egg, deutovum, larval, three nymphal, and one adult stages. *B. depressa* reared on clover mites and two-spotted spider mites at 60° F. and 90% relative humidity developed from larvae to adults in 21 to 30 days, while those reared at 70° F. and 90% R.H. required only 14 to 21 days. Attempts to rear mites at temperatures higher or lower than these failed. Due at least in part to certain similarities in their life cycles *B. depressa* was an important predator of the clover mite.

ERRATUM

On page 631 of Vol. 49, No. 5 in the fourth line from the bottom of column 1 the word "minutes" should read "months."

Phosphorus Compounds as Ovicides and Adulticides against Body Lice[1]

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The use of organic phosphorus insecticides has become standard practice for the control of many arthropod pests that are resistant to DDT or other chlorinated hydrocarbons. Since 1952 many phosphorus compounds have been tested at Orlando, Fla., for possible use against the body louse (*Pediculus humanus humanus* L.). Blanton (1953) showed that parathion was slightly less effective than heptachlor, dieldrin, or lindane in louse powders. King (1954) reported the results of screening tests with about 200 phosphorus compounds, of which 26 were outstanding as adulticides and 4 as ovicides against body lice. Recently Smith & Avens (1954) reported on the ovicidal action of parathion against the peach tree borer. Eddy *et al.* (1954) found several compounds effective as synergists with the phosphorus insecticides Potasan, Bayer 21/199 and 21/200 against body lice. Fluno (1955) reported tests with certain chlorinated hydroxyethylphosphonates and vinyl phosphates against lice and other species. This paper reports studies conducted in 1954 and 1955 to evaluate 73 phosphorus compounds as ovicides and adulticides against body lice.

Materials and Methods.—The eggs and adult lice used in most of the tests were from the regular nonresistant laboratory colony. In a special test for resistance the lice were from the extremely DDT-resistant Korean strain described by Eddy *et al.* (1955). The eggs ranged in age from fresh to 2 days old, except in special tests to determine the effect of age on the action of ovicides, and the adults were 1 to 3 days old at the time of testing.

All the compounds were tested in acetone solutions or pyrophyllite powders. The ovicide tests with solutions were conducted according to the methods described by Eddy (1944) and those with powders according to Bushland *et al.* (1944) with a few modifications. Acetone was used as a solvent instead of alcohol as used by Eddy. The number of eggs was 50 to 100. The cloth pads containing eggs were treated by shaking in a 4-ounce jar containing approximately 10 grams of powder. Studies were made with pads exposed to a range of humidities in glass humidifiers containing various concentrations of sulfuric acid, as described by Solomon (1951). All tests, including those in the humidifiers, were conducted in an air-conditioned room at 80° F. and 60 to 70% relative humidity.

Tests against adult lice were conducted according to the methods described by Eddy *et al.* (1954), but were run in the air-conditioned room mentioned above.

Results.—*Comparative ovicidal and adulticidal effectiveness in solutions.*—The minimum concentrations (MLC) of each compound in acetone solution required to cause complete mortality of eggs and adult lice are listed in table 1, in descending order of the ovicidal effectiveness. None of the compounds were tested at a concentration higher than 1%.

Parathion (0-15108) was the most effective material as an ovicide and sulfotepp (0-16273) as an adulticide. Malathion (0-17034) and Chlorthion (0-18861), which hold the most promise as louse powders because of their low mammalian toxicity, were also highly effective against both eggs and adults.

Ovicidal effectiveness of powders.—Ovicide tests were conducted with malathion and Chlorthion at several concentrations in pyrophyllite powders. In triplicate tests the average mortalities of eggs at concentrations of 1, 0.5, and 0.25% were 100, 97.6, and 38.6%, respectively, with malathion, and 96.7, 65.9, and 21.1% with Chlorthion.

Effectiveness of synergists for ovicides.—Eddy (1944) reported *N*-isobutylundecenamide (IN-930) to be a synergist for dinitroanisole as a louse ovicide. Four phosphorus compounds that were synergized by sulfoxide in previous tests against adult lice (Eddy 1954 and unpublished data) were tested at various concentrations alone and in combination with sulfoxide to determine whether their ovicidal action was also synergized.

The addition of sulfoxide increased the effectiveness of *O,O′,O″,O‴*-tetraethyl thiopyrophosphate (0-19131), but not that of *O,O*-diethyl *O*-*p*-nitrophenyl phosphate (0-16087), *O,O*-diethyl *O*-(4-methylumbelliferone)phosphorothioate (0-17296), or *O*-(3-chloro-4-methylumbelliferone) *O,O*-dimethyl phosphorothioate (0-19196). The results with the first compound are presented in table 2.

Resistance to ovicides and insecticides.—Resistance of lice to DDT has been reported by Hurlbut *et al.* (1952, 1954). Eddy *et al.* (1955) reported the development of resistance to DDT but not to lauseto neu, lindane, and pyrethrins in laboratory colonies. Resistance of louse eggs to an ovicide (lauseto neu) was reported by Cole (1955). Parathion, malathion, TEPP, Diazinon, EPN, Potasan, Systox, para-oxon, Chlorthion, and sulfotepp were tested in acetone solutions against eggs and adult lice from DDT-resistant and nonresistant colonies. There was no difference between the two colonies.

Influence of humidity on the ovicidal action of malathion.—Malathion was selected for tests of the influence of humidity on ovicidal action. Hansens (1944) and Eddy (1947) had shown that the effectiveness of certain ovicides against eggs of body lice varied with the humidity, but no phosphorus compounds were included.

The results of tests with six concentrations of malathion in acetone solutions at several relative humidities, given in table 3, show a close correlation between the relative humidity and the ovicidal effectiveness. Complete mortality of eggs at all relative humidities was obtained only at the highest concentration (0.05%). At 90% relative humidity the mortality was high at all concentrations and in the untreated check. Studies by Mellanby (1932) and Marsh & Buxton (1937) indicate that the relative humidity under clothing usually will not exceed 70%. At this relative humidity 0.025% malathion gives high but incomplete kill of eggs.

[1] This work was conducted at the Orlando, Fla., laboratory of the Entomology Research Branch under funds allotted by the Department of the Army. Accepted for publication May 4, 1956.

[2] The authors acknowledge the assistance of D. L. Van Natta in conducting the tests.

Table 1.—Minimum lethal concentrations of various compounds as ovicides and adulticides against body lice.

OVICIDE MLC (%)	COMPOUND	ORLANDO NO.	ADULTICIDE MLC (%)
0.0025	Phosphorothioic acid, *O,O*-diethyl *O*-(*p*-nitrophenyl) ester (parathion)	15108	0.00005
.005	Phosphoric acid, diethyl *p*-nitrophenyl ester (para-oxon)	16087	.0005
.01	Phosphorothioic acid, *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) ester (Diazinon)	19507	.00001
.025	Dithiopyrophosphoric acid, *O,O',O'',O'''*-tetraethyl ester (sulfotepp)	16273	.0000025
.05	Phosphorodithioic acid, *O,O*-diethyl *O*-(isopropylthiomethyl) ester (Am. Cyanamid 12009)	22866	.0001
	Phosphorothioic acid, *O,O*-diethyl *O*-(2-ethylthioethyl) ester (Systox)	17295	.01
	Succinic acid, mercapto-, diethyl ester, *S*-ester with *O,O*-dimethyl phosphorodithioate (malathion)	17034	.001
.1	Phosphoric acid, 1-carbomethoxy-1-propen-2-yl diethyl ester (Shell OS 1808)	22375	.005
	Phosphorothioic acid, *O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl ester (Chlorthion)	18861	.001
	Phosphorodithioic acid, *O,O*-diethyl *O*-(isopropylthiomethyl) ester (Am. Cyanamid 12008)	22865	.000025
	Phosphorothioic acid, *O,O*-diethyl *O*-(6-methyl-2-propyl-4-pyrimidinyl) ester (Pirazinon)	22820	.0001
	Dithiopyrophosphoric acid, *O,O',O'',O'''*-tetrapropyl ester (NPD)	16894	.00005
.25	Phosphonothioic acid, phenyl-, *O*-ethyl *O*-*p*-nitrophenyl ester (EPN)	17798	.00025
	Phosphorothioic acid,		
	O-cyclohexyl *O,O*-diethyl ester	20507	.0005
	O-cyclohexylmethyl *O,O*-diethyl ester	20647	.0005
	O,O-diethyl *O*-tetrahydro-2-furfuryl ester	20540	.0005
.5	Phosphoramidothioic acid, *N*-(1-carbethoxyethyl)-, *O,O*-diethyl ester	20722	.00005
	Phosphorothioic acid, *O*-3,4-dichlorobenzyl *O,O*-diethyl ester	17478	.0005
1	Phosphoramidothioic acid,		
	N-(2-carbethoxyethyl)-, *O,O*-diehtyl ester	20669	.001
	N-carbethoxymethyl-, *O,O*-diethyl ester	20657	.001
	N-(1-carbethoxypropyl)-, *O,O*-diethyl ester	20703	.00025
	N-(2-carbethoxypropyl)-, *O,O*-diethyl ester	20704	.0001
	Phosphoric acid, 1-carbomethoxy-1-propen-2-yl dimethyl ester (Shell OS 2046)	22374	.0025
	Phosphorothioic acid,		
	O-(*alpha*-allyl-*o*-methoxybenzyl) *O,O*-diethyl ester	20652	.001
	O-(*alpha*-allylpiperonyl) *O,O*-diethyl ester	20502	.001
	O-benzyl *O,O*-diethyl ester	20504	.0005
	O-(*alpha*-carboallyloxy)benyzl *O,O*-diethyl ester	20654	.0005
	O-(1-carbethoxyethyl) *O,O*-diethyl ester	20706	.0001
	O-(3-carbethoxy-1-methylpropyl) *O,O*-diethyl ester	20673	.0025
	O-(*p*-chloro-*alpha*-propylbenzyl) *O,O*-diethyl ester	20566	.0025
	O-cyclohexyl *O,O*-diethyl ester	20507	.001
	O,O-diethyl *O*-(7,7-dimethylbicyclo[3.1.1]hept-2-ene-2-ethyl) ester	20674	.0025
	O,O-diethyl *O*-*p*-isopropylbenzyl ester	20650	.0005
	O,O-diethyl *O*-(*p*-methoxybenzyl) ester	20499	.001
	O,O-diethyl *O*-(4-methylumbelliferone) ester (Potasan)	17296	.001
	O,O-diethyl *O*-phenethyl ester	20541	.0005
	O,O-diethyl *O*-5-phenylpentyl ester	20653	.0025
	O,O-diethyl *O*-(*alpha*-propylanisyl) ester	20563	.001
	O,O-diethyl *O*-*o*-veratryl ester	20505	.0025
	Pyrophosphoric acid, tetraethyl ester (TEPP)	16796	.00025
	Thiopyrophosphoric acid, *O,O',O'',O'''*-tetraethyl ester	19131	.0005
>1	Amidophosphoryl fluoride, *N,N,N',N'*-tetramethyldi- (Dimefox, Hanane)	19109	.0025
	Phenothiazine, *N*-(diethoxyphosphinothioyl)-	20692	.0025
	Phosphonic acid,		
	p-chlorophenyl-, diethyl ester	19127	.25
	N,N-diethylcarbamyl-, *O,O*-diethyl ester	20670	.25
	ethyl-, *O*-(*N,N*-diethylcarbamyl) *O*-ethyl ester	20671	.1
	octyl-, diethyl ester	18569	.25
	2,2,2-trichloro-1-hydroxyethyl-, diethyl ester	17763	.25
	2,2,2-trichlorohydroxyethyl-, *O,O*-dimethyl ester (Bayer L 13/59)	19763	.0025
	Phosphoramido thioic acid,		
	N-carbethoxymethyl-, *O,O*-dimethyl ester	20693	.01
	N-(2-carbethoxypropyl)-, *O,O*-dimethyl ester	20705	.1
	Phosphoric acid,		
	2-chlorovinyl diethyl ester (Shell OS 1836)	22373	.0025
	2,2-dichlorovinyl dimethyl ester (DDVP)	20738	.005
	diethyl *N,N*-diethylcarbamyl ester	20672	.01
	Phosphorothioic acid,		
	O-allethrolonyl *O,O*-diethyl ester	20500	.0025
	O-benzhydryl *O,O*-diethyl ester	20508	.01
	O-[1,1-bis(*p*-chlorophenyl)ethyl] *O,O*-diethyl ester	20664	.01
	O-2-chlorobenzyl *O,O*-diethyl ester	20565	.0025
	O-(3-chloro-4-methylumbelliferone) *O,O*-diethyl ester	17957	.00025
	O-(3-chloro-4-methylumbelliferone) *O,O*-dimethyl ester	19196	.005

Table 1. (Continued)

OVICIDE MLC (%)	COMPOUND	ORLANDO NO.	ADULTICIDE MLC (%)
>1	Phosphorothioic acid,		
	O-(2-chloro-4-nitrophenyl) *O,O*-dimethyl ester	17035	.0001
	O-3-(*o*-chlorophenyl)propyl *O,O*-diethyl ester	20648	.001
	O-3-(*p*-chlorophenyl)propyl *O,O*-diethyl ester	20649	.001
	O-(*o*-chloro-*alpha*-propylbenzyl) *O,O*-diethyl ester	20651	.0005
	O-(4,4′-dichlorobenzylhydryl) *O,O*-diethyl ester	20567	.01
	O-2,4-dichlorophenyl *O,O*-diethyl ester	17470	.005
	O,O-diethyl *O*-hydrocinnamyl ester	20539	.0025
	O,O-diethyl *O*-2-(1-naphthyl) ethyl ester	17491	.025
	O,O-diethyl *O*-1-naphthylmethyl ester	17487	>1
	O,O-diethyl *O*-[*alpha*-(3-phenylpropyl)-piperonyl] ester	20564	.0025
	O,O-diethyl *O*-(*alpha*-propylbenzyl) ester	20562	.0025
	O,O-diethyl *O*-veratryl ester	20506	.001
	Pyrophosphoramide, octamethyl- (schradan)	17291	.001

Effect of age of eggs.—Tests were conducted to determine the relative effectiveness of malathion, Chlorthion, and dinitroanisole against eggs of different ages. Dinitroanisole has been used in louse powders as an ovicide (Bushland *et al.* 1944), and Hansens (1944) and Eddy (1947) showed that young eggs were more suceptible than older eggs to dinitroanisole and other compounds, but no phosphorus compounds were tested.

The results of tests with three concentrations of each ovicide are given in table 4. Dinitroanisole was most effective against the youngest eggs (1-day-old), as in the studies of Hansens and Eddy, but with the phosphorus compounds the mortality became progressively higher as the age of the eggs increased. At a concentration of 0.025%, malathion caused mortalities ranging from 50.5% against 1-day-old eggs to 94.4% against 6-day-old eggs, and Chlorthion caused mortalities ranging from 14.7 to 83.5%, respectively.

Effectiveness of powders against adult lice.—Results of tests with malathion and Chlorthion at several concentrations in powders are presented in table 5. Both materials at 0.1% were completely effective for 14 days and over 90% effective after 21 days. Chlorthion appeared to be slightly more effective than malathion.

Table 2.—Effectiveness of sulfoxide as a synergist for the ovicide *O,O′,O″,O‴*-tetraethyl thiopyrophosphate.[a]

PER CENT CONCENTRATION		PER CENT MORTALITY
Ovicide	Sulfoxide	
0.1	1.0	100
.05	.5	100
.025	.25	25.4
.01	.1	19.9
.1	0	49.8
.05	0	25.3
.025	0	22.3
.01	0	9.1
0	1.0	17.6
0	.5	16.7
0	.25	15.9
0	.1	15.2
Untreated eggs		10.1

[a] Two replications.

Table 3.—Per cent mortality of body louse eggs exposed to various concentrations of malathion at various relative humidities.

CONCENTRATION (PER CENT)	RELATIVE HUMIDITY (PER CENT)						
	30	50	70	75	80	85	90
0.05	100	100	100	100	100	100	100
.025	70.2	87.8	96.2	95.1	98.8	100	100
.01	25.9	49.0	58.7	52.2	79.0	96	100
.005	11.4	14.8	34.5	32.8	55.3	86.1	98.7
.0025	9.2	7.3	20.4	18.7	29.5	48.1	99
.001	10.2	11.2	15.9	11.7	20.8	30.8	88.5
Untreated	7.8	5.8	8.3	14.1	22.5	24.9	95

DISCUSSION.—Phosphorus insecticides are not yet recommended for body louse control. Although toxicologica data now available indicate that some may be as safe to use on warm-blooded animals as lindane or DDT, further tests must be performed before their toxicity to man will be known. If some phosphorus insecticides are found safe for use on man, further studies should be made against body lice on the human host under practical conditions.

SUMMARY.—Seventy-three phosphorus compounds were tested as ovicides and adulticides against laboratory

Table 4.—Effectiveness of malathion, Chlorthion, and dinitroanisole against body louse eggs of different ages.[a]

OVICIDE AND CONCENTRATION (PER CENT)	PER CENT MORTALITY AFTER DAYS SHOWN					
	1	2	3	4	5	6
Malathion						
0.05	100	100	100	100	100	100
.025	50.5	54.3	64.7	80.7	83	94.4
.01	17	17.7	18.6	23.7	28.5	40
Chlorthion						
0.1	100	100	100	100	100	100
.05	68.8	66.3	93.9	92.1	95.5	94.2
.025	14.7	16.6	21.7	37.8	64.8	83.5
Dinitroanisole						
10	98.5	97.3	91.1	94.4	91.2	97.5
1	96.3	94	94	94.8	80.9	91.3
.1	93	80	66.4	71.6	61.9	72.7
Untreated	8.4	11.3	5.4	8	7.8	4.7

[a] Two replications.

Table 5.—Effectiveness of malathion and Chlorthion powders against adult lice.[a]

Insecticide and Concentration (Per Cent)	Per Cent Knockdown After 1 Hour	Per Cent Mortality After— 0 day	7 days	14 days	21 days
Malathion					
0.1	100	100	100	100	92.5
.05	70	100	100	92.5	82.5
.025	0	100	87.5	60	—
.01	0	52.5	—	—	—
.005	0	17.5	—	—	—
Chlorthion:					
0.1	100	100	100	100	95
.05	65	100	100	97.5	75
.025	0	100	100	72.5	35
.01	0	100	45	—	—
.005	0	75	—	—	—
Checks:					
Pyrophyllite	0	2.5	0	2.5	2.5
Untreated	0	0	0	0	2.5

[a] Two replications.

colonies of body lice (*Pediculus humanus humanus* L.). In acetone solutions parathion was the most effective ovicide, causing complete kill of eggs at a concentration of 0.0025%, and sulfotepp was the most effective against adult lice, causing complete kill at 0.000025%. Malathion and Chlorthion, which hold some promise for practical use in louse powders because of their low mammalian toxicity, were completely effective as adulticides at 0.001% and as ovicides at 0.05 and 1%, respectively. Malathion and Chlorthion in powders were about 97% effective as ovicides at 0.5 and 1%, respectively. The ovicidal action of *O,O',O'',O'''*-tetraethyl thiopyrophosphate was synergized by the addition of sulfoxide, but that of three other phosphorus compounds was not. Lice and eggs from a colony highly resistant to DDT showed no cross-resistance to 10 phosphorus compounds. The ovicidal effectiveness of malathion was directly correlated with the relative humidity, and that of malathion and Chlorthion with the age of the eggs. Pyrophyllite powders containing 0.1% of malathion or Chlorthion were completely effective against adult lice for 14 days.

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Bait Stations for the Control of House Flies[1]

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The effectiveness of liquid baits for the control of house flies (*Musca domestica* L.) has been reported by Thompson *et al.* (1953), Gahan *et al.* (1954a), and Langford *et al.* (1954), of dry sugar baits by Gahan *et al.* (1954b), and of granular baits by Keller and Wilson (1955). The advantages of these baits are that most of the flies find and feed on them in a matter of minutes, and there is no hazard to animals or children if they have been properly applied. Very little insecticide and no special apparatus are required. However, these baits must be applied at 1- to 3-day intervals until breeding pressure is sufficiently reduced to permit less frequent applications. There is a considerable loss of liquid baits when they are applied to absorbent surfaces, and granular and sugar baits soon become covered when used on soil, trash, or litter. All three kinds may be swept or washed away when used on floors in buildings.

These shortcomings suggested the desirability of methods of application that minimize loss and give fly control for protracted periods with a single treatment. Bruce (1953), and others have obtained success in screened buildings with thick sirup baits painted on selected surfaces and allowed to harden to a "varnish." In our experience treatments of this type have given satisfactory control when applied to posts and fences in feed lots or holding pens, but in many outdoor situations there are no suitable sites for application, and in dairy barns owners complained because the residue turned dark, and showed through several coats of water-base paint.

Methods and Materials.—Experiments were therefore conducted with bait stations designed to combine the best features of the scattered baits with a more permanent type of application. The bait stations were prepared by stapling wooden tongue depressors to absorbent paper coasters $3\frac{1}{4}$ inches in diameter, 4- by 4-inch pieces of screen, or 4- by 7-inch pieces of plastic-impregnated screen the top 3 inches of which was bent down at an acute angle to provide a protective awning. The coasters were soaked for 24 hours in honey containing 2% of malathion, or coated with 25 grams of melted milk chocolate containing 2% of malathion. Each screen station was coated on one side with approximately 25 grams of a bait slurry containing the insecticide, sugar, and sometimes agar, sand, or other ingredients. To facilitate finding and feeding by flies, the stations were generally placed with the treated portion as near to the ground or floor as possible, where flies were feeding, breeding, or congregating. It is desirable to keep the stations in as nearly a vertical position as possible to prevent dead flies from obstructing access to the bait, and to place the baits where animals cannot feed on them.

House fly populations were determined from the number of flies feeding on cloth strips (1 by 24 inches) saturated with a 50% malt-water solution. The effectiveness of the treatments was based on the difference in counts before and at various intervals after treatment.

Results.—Experiments with the different bait formulations under various conditions during the summer of 1955 are summarized in table 1. All baits contained 2% of the insecticide except No. 3, which contained 1%. The other ingredients were as follows (figures in per cent): (1) agar 2, sand 46, sugar 50; (2) agar 2, sugar 96; (3) sugar 99; (4) honey 98; (5) milk chocolate 98; (6) sugar 98; (7) honey 50, powdered milk 48; (8) sugar 50, powdered milk 48; (9) agar 2, peanut oil 2, sugar 94. Except as noted the tests were conducted near Orlando, Fla.

In dairy barns the stations were stapled to door frames, propped against walls, or placed on ledges or window sills, and in the feed rooms the stations were placed on empty and full feed sacks, feed carts, and pallets. Occasionally they were suspended from wires or strings on pull-chain light fixtures.

The observations in dairy barns indicated a great immediate reduction, and this reduction continued until the toxicity was lost or the stations were destroyed or damaged by personnel, animals, or water. Five to forty bait stations containing Bayer L 13/59 caused reductions between 85 and 99% after 1 day, and except in one test the reduction was above 85% for 28 to 70 days. Fifteen to fifty bait stations containing malathion were 95 to 96% effective after 1 day, and the reduction ranged from 67 to 99% for 42 to 70 days.

In poultry houses containing caged laying hens the bait stations were used on the ground at the rate of one station every 10 feet of cage. They were placed at the edge of the manure and at right angles to the line of cages by pushing the tongue depressor into the manure. Fifty to one hundred bait stations containing Bayer L 13/59 gave reductions ranging between 73 and 98% after 1 day and between 88 and 99% for 42 to 84 days. There was little difference between the various formulations. Stations prepared with bait No. 1 containing malathion were 82 to 84% effective after 1 day, and three of the four were 89 to 99% effective for 21 to 98 days. In single tests bait stations prepared with chocolate, honey, honey and powdered milk, or sugar alone were less effective than those prepared with sand, sugar, and agar. Stations prepared with bait No. 8 containing Am. Cyanamid 4124 gave 85% reduction 1 day after treatment and 93 to 99% for 28 days.

At hog pens the bait stations were stapled or otherwise placed about the garbage rack and cooker, stapled to fence posts, or stuck into the soil or manure outside the pens. Two tests with a hundred Bayer L 13/59 stations gave reductions of 91 to 99% for 21 and 84 days. Malathion stations gave reductions of 0 to 99% after 1 day, and subsequent control ranged from very poor to excellent for as long as 98 days. Two tests with Chlorthion stations were moderately effective, giving reductions between 73 and 97% for 21 and 28 days.

In feed lots the stations were placed in feed rooms or passageways, stapled to fence posts, feed bunks, or other vertical surfaces, or pushed into the ground or manure away from regular traffic by animals. Bayer L 13/59 gave 93 to 98% control from the first to the 21st or 42nd day

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Table 1.—Control of house flies in various habitats with bait stations.

Bait No.	Average Pre-treatment Count	Number of Stations	Per Cent Reduction After Indicated Days							
			1	3–4	7	14	21	28	42	56
Dairy Barns										
Bayer L 13/59										
1	205	35	97	99	98	99	98	97	99	94[a]
1	113	32	96	99	98	98	96	87	91	87
1	99	30	99	97	97	98	98	98	94	92[a]
1	124	40	85	98	98	95	98	98	98	96
1	169	20	99	99	97	98	99	97	71	—
1	500+	10	93	89	99	97	87	96	—	—
1	80	5	90	93	98	60	—	—	—	—
1	117	8	93	98	97	92	98	98	—	—
Malathion										
1	203	15	95	98	99	73	99	98	99	46
1	74	25	96	98	97	98	98	97	95	93[a]
1	116	50	96	93	96	98	67	93	79	73
Poultry Houses										
Bayer L 13/59										
1	148	100	92	98	99	99	99	97	96	91[a]
2	78	100	74	95	94	—	99	92	96	48
2	64	100	86	97	88	94	99	91	95	95[a]
3	179	75	98	98	99	99	99	99	99	62
1	98	50	73	99	99	99	99	98	—	—
1	108	75	98	99	98	99	98	99	93	97[b]
Malathion										
4	50	76	4	98	38	56	—	—	—	—
5	51	50	35	96	94	82	88	94	98	98[a]
6	32	76	88	99	89	93	80	54	95	0
1	141	150	—	99	98	99	99	99	99	98[c]
1	86	75	—	98	98	98	98	97	90	75[c]
1	69	50	84	89	94	85	96	97	55	—
1	65	75	82	99	99	92	92	—	—	—
7	75	131	—	71	91	80	56	—	—	—
Am. Cyanamid 4124										
8	98	50	85	99	99	99	99	93	62	90
Hog Pens										
Bayer L 13/59										
2	330	100	93	96	99	96	99	99	99	98[b]
3	416	100	94	97	97	94	91	33	—	—
Malathion										
2	144	100	95	92	87	58	27	—	—	—
6	225	75	99	97	94	97	99	93	92	99[c]
6	460	75	99	99	99	99	99	97	99	99[c]
2	79	50	—	0	10	18	86	87	66	—
1	230	50	—	87	89	72	88	90	—	—
2	312	80	81	90	81	90	92	94	62	—
1	141	100	16	91	67	—	—	—	—	—
2	14	5	72	0	86	79	50	50	79	57
1	310	50	27	92	97	99	97	98	35	—
1	124	50	0	7	3	0	52	0	—	—
Chlorthion										
9	280	50	70	97	94	74	96	0	—	—
9	179	50	69	73	89	79	93	79	39	—
Feed Lots										
Bayer L 13/59										
3	462	100	90	40	24	99	96	97	70	54
6	514	124	93	96	96	94	94	74	—	—
1	215	50	95	98	95	98	97	95	97	45
Kansas Farms										
Bayer L 13/59										
1	124	79	69	82	—	96	--	—	—	—
1	128	88	82	99	—	99	—	—	—	—
Malathion										
1	115	58	77	94	—	96	—	—	—	—
1	76	68	78	79	—	96	—	—	—	—
Nebraska Farms										
Bayer L 13/59										
1	66	67	16	73	97[d]	—	—	—	—	—
1	81	78	58	73	97[d]	—	—	—	—	—
1	86	127	81	89	94[d]	—	—	—	—	—
Malathion										
1	69	69	46	89	97[d]	—	—	—	—	—
1	214	214	22	87	65[d]	—	—	—	—	—

[a] Greater than 90% after 70,[a] 84[b] or 98 days.[c]
[d] After 5 days.

in two tests, whereas in the third test control was poor the first week but excellent for the next 3 weeks.

On farms in Kansas and Nebraska most of the stations were stapled to mangers, studs, plates, doors, and other vertical surfaces inside animal sheds, barns, and milking rooms. An occasional station was placed outside where flies were congregating about unsanitary situations. Malathion and Bayer L 13/59 were about equally effective. Control ranged from 16 to 82% after 1 day and required 2 to 5 days to reach 90% in six tests and 11 days in two tests. In one test the control did not reach 90% during the 5 days of observation.

In general, bait stations containing Bayer L 13/59 or malathion gave excellent control of flies where a sufficient number of stations were used and sanitation was at least fair. Stations containing Chlorthion or Am. Cyanamid 4124 gave good control in a limited number of tests. In some tests a high degree of control was not obtained as quickly as in previous tests with scattered baits, and control was not obtained as quickly in Kansas and Nebraska as in Florida.

SUMMARY.—Experiments for the control of house flies (*Musca domestica* L.) were conducted in dairy barns, poultry houses, hog pens, and feed lots in Florida, and on farms in Kansas and Nebraska with bait stations designed to combine the best features of scattered baits with a more permanent type of application. The bait stations consisted of wooden tongue depressors stapled to paper coasters or pieces of screen coated with a slurry containing an insecticide, sugar, and sometimes agar, sand, or other ingredients.

In general, baits containing 2% of Bayer L 13/59 or malathion gave excellent control where a sufficient number of stations were used and where sanitation was at least fair. Both Chlorthion and Am. Cyanamid 4124 at 2% gave good control in a limited number of tests. The baits were effective for 28 to 98 days.

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Protection of Stored Corn with Lindane-Impregnated Sawdust[1]

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The actual loss, and the progressive decrease in feeding quality, of grain, especially corn, in Louisiana caused by stored grain insects is overwhelming. The exact damage over a year's storage has never been determined. Estimates have ranged from a minimum of 10% upward. Studies to determine initial and progressive losses to corn in storage on the farm in Louisiana are now under way along with this study. Preliminary investigations made in 1955 by the writers from corn samples collected at harvest from the various ecological areas of the state have revealed that initial damage at harvest time varied from less than 1% to an average of 27%, dependent on the area. Average damage for the state at harvest time was 8.7% The species which were found to be responsible for this damage originating in the field were, in order of importance, the rice weevil, *Sitophilus oryza* (L.), pink cornworm, *Pyroderces rileyi* (Wals.), square-necked grain beetle, *Cathartus quadricollis* (G.-M.), confused flour beetle, *Tribolium confusum* Duv., saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and the Angoumois grain moth, *Sitotroga cerealella* (Oliv.). Some samples contained more than 90% of the kernels damaged. The rice weevil was found to be responsible for the major portion of the damage. Individual ears were found before harvest which contained as many as 150 adult weevils. The advisability of storing corn containing such infestations is very questionable. Farmers are usually advised to grind and feed the grain as quickly as possible. Under conditions of such heavy initial infestations, it is readily seen that under the mild temperatures the year round in South Louisiana, the development of these insects will continue after storage, and in a relatively short time the quality of the grain will be extremely low. The lower mean temperature in North Louisiana and the normally lower field infestation make it possible to carry corn over winter and well into the summer before the loss is too great in that part of the state.

Corn is stored in Louisiana in wooden cribs. Cracks are often purposely left between boards. The floors are usually tight. This kind of crib cannot be fumigated effectively by the farmer. Fumigation has been recommended and used but the results have never been satisfactory.

The importance of corn to the South and especially to Louisiana is increasing as livestock production expands. More than 50% of the acreage is now planted to the recommended hybrids, and with new techniques in cultivation and fertilization and soil insect control, yields have increased one-third in the last 10 years. The storage of the corn produced and its protection from insect damage have not kept pace with the new production practices. In addition, the use of mechanical pickers which shuck clean or partially shuck a high percentage of the ears in the field is presenting an additional problem in insect control. The ears are opened for attack by the Angoumois grain moth the damage by which, in the past, has been confined largely to shelled grain and to ears with inadequate shuck coverage.

With the above points in mind it appeared clear that the logical immediate approach to the problem was through the development and use of a continuous type of protectant which would remain effective from one harvest period to the next. The synergized pyrethrums were investigated. They offered a certain degree of protection, but their residual quality disappeared under South Louisiana conditions after approximately 6 to 7 months. The degree of protection obtained through their use was apparently dependent on the initial infestation and the size of the storage bin. The lower the initial infestation and the larger the bin, the greater the protection. The first test in this study, in which lindane was absorbed on broken bits of corn cobs and used as a protectant for corn, was conducted concurrently with tests using synergized pyrethrum, (Floyd & Smith 1953). These first tests were set up on shucked corn at approximately 60 p.p.m. After 2 years this corn was in the same condition as when stored. Corn in untreated bins was totally destroyed after 10 months. Subsequent investigations concerning control of stored grain insects in farm-stored corn have been directed largely towards the development and refinement of a technique using lindane.

Procedure.—Experiments previous to the 1954–1955 storage season were directed towards techniques of impregnation, minimum dosage levels required to effect control for a 12 months' storage period, effect of distribution of the treatment in the corn mass, and effects of the treatment under different conditions of corn dressing, i.e., snapped, shucked, and shelled. Dry pine sawdust was found more effective than corn cobs as a carrier for lindane for this purpose. Its absorptive capacity is such as to render possible the preparation of almost any concentration desired.

The actual preparation of the treatment was as follows: technical lindane was dissolved in xylol or acetone. A 10 to 20% solution is satisfactory. This solution was sprayed from a fan-type nozzle under low pressure on the sawdust inside a mixer. After the required amount of liquid had been applied, the mixture was removed and spread out in the shade to dry. Mixtures prepared from acetone solutions dry in about one-tenth the time required for mixtures prepared from xylol solutions. After the sawdust was completely dry it was ready for use. For experimental purposes a 5% mixture has been found quite satisfactory for both laboratory and farm crib tests. Concentrations ranging from 1 to 20% have been prepared and used.

One 20-bushel load of corn required approximately ½ pint of a 5% mixture to obtain a treatment equivalent to approximately 10 p.p.m. All dosage rates were calculated on the basis of shelled corn at 56 pounds per bushel regardless of the corn dressing, i.e., snapped corn (slip shucked) weighs approximately 70 to 72 pounds per bushel. Unless otherwise stated, all tests were conducted on Dixie 18 corn.

Laboratory tests on shelled corn—1954–1955 season.

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Table 1.—Effect of dosage rate of lindane from impregnated sawdust in the protection of shelled corn from damage by stored grain insects.[a]

Concentration (p.p.m.)	Per Cent Kernels Damaged After		
	7 Mo.	10 Mo.	13 Mo.
4	1.0	1.0	1.0
1	1.5	2.0	2.5
0.5	0.8	1.8	1.5
0.1	1.3	3.5	43.0
Check	16.5	50.5	99.0
L.S.D. at 5% level	6.6	10.9	13.0
at 1% level	9.2	15.3	18.0

[a] The initial damage count of this corn was 1%. The rice weevil and Angoumois grain moth were responsible for the majority of the damage.

Small-scale laboratory tests were conducted on shelled corn in 3-pound lots stored in ½-gallon fiber containers with ¼-inch mesh screen over the tops. Storage was in a grain room maintained at room temperature. A heavy infestation of stored grain insects was present in this room at all times. Treatments were replicated four times. Three examinations were made over a 13 months' storage period (table 1).

An additional laboratory test on shelled corn was conducted using wooden boxes 1 cubic foot in volume as the containers equipped with ¼-inch mesh screen tops. These boxes were stored in an open-type building. In order to determine the value of tight construction, part of these boxes were "ventilated," having ½-inch holes (11) drilled in each box—3 in the bottom and 2 on each side.

Treatments were applied on each 6-inch rise of the grain as the boxes were filled. Treatments were replicated four times. A single examination was made after 12 months' storage (table 2).

Laboratory tests on snapped corn.—A series of tests was conducted on slip-shucked corn treated at various dosage levels and stored in 55-gallon steel drums equipped with ¼-inch mesh screen tops. All containers rested on 2-inch blocks. Some of the drums were ventilated, each having six ½-inch holes drilled in the side and six in the bottom. The purpose was to determine the minimum dosage required to effect control of stored grain insects in snapped corn and the necessity for tight construction in obtaining this control in relation to dosage rate. Each drum contained 2 bushels of corn. Treatments were replicated three times. The storage period was approximately 12 months in a common crib-type structure with ventilation from four sides. A single examination was made after 12 months' storage (table 3).

Laboratory tests on shucked corn.—A similar type test as described above was conducted on shucked corn. These drums were stored in the same building with the snapped corn. Treatments were replicated three times. Each replicate contained 2.8 bushels. A single examination was made after 12 months' storage (table 4).

Effect of the treatment on insect development in the grain after removal of the treated sawdust from the grain.—Uninfested shelled corn was placed in 50-pound lard cans and treated with 10 p.p.m. of 5% lindane-sawdust. After 3 months the grain and sawdust were separated by screening. Separate containers of similar corn were kept fumigated and closed for this 3-month period. Grain from these

Table 2.—Effect of dosage rate of lindane in the protection of shelled corn in tight versus ventilated containers.[a]

Concentration (p.p.m.)	Per Cent Kernels Damaged After 12 Months[b]	
	Tight Box	Ventilated Box
4	1.0	1.0
1	5.0	6.0
0.5	19.3	23.3
1[c]	5.0	(not used)
Check	91.0	—
L.S.D. at 5% level	17.2	
at 1% level	24.2	

[a] Containers were constructed of plywood and measured exactly one cubic foot in volume. The tops were made of ¼-inch screen. "Ventilated" refers to 2½-inch holes drilled in each side and 3 holes in the bottom of each—making a total of 11 one-half inch holes per box.
[b] The initial damage in this corn was 1%.
[c] Fumigated corn used to fill boxes.

Table 3.—Effect of dosage rate of lindane in the protection of snapped corn at different levels in the test containers.[a]

Concentration (p.p.m.)	Per Cent Kernels Damaged After 12 Months[b]			Responsible Species[c]	
	From Top Half	From Bottom Half	Mean Damage	Top Sample	Bottom Sample
Unventilated drum					
4	1	1	1.0	A	A
1	6	1	3.5	B	A
Ventilated drum					
4	1	1	1.0	A	A
1	5	3	4.0	B	A
Check	23	51	37.0	A	A
L.S.D. at 5% level			13.6		
at 1% level			19.7		

[a] 55-gallon steel drums—¼-inch mesh screen tops. Ventilated drums contained 6 one-half inch holes in the side and 6 in the bottom of each.
[b] The initial damage of this corn was 1%.
[c] A, rice weevil; B, Angoumois moth.

Table 4.—Effect of dosage rate of lindane in the protection of shucked corn at different levels in the test containers.[a]

Concentration (p.p.m.)	Per Cent Kernels Damaged After 12 Months[b]		
	From Top Half	From Bottom Half	Mean Damage
Unventilated drum			
4	72.4	2.8	37.6
1	97.7	55.2	76.4
Ventilated drum	86.9	26.8	56.8
4			
1	98.8	93.9	96.3
Check	99.5	99.7	99.6
L.S.D. at 5% level			25.5
at 1% level			39.6

[a] 55 gallon steel drums—¼-inch mesh screen tops.
[b] The initial damage of this corn was 3%. Angoumois grain moth responsible for all damage except in bottom half of unventilated drum treated at 4 p.p.m. where the rice weevil was responsible.

Table 5.—Effect of lindane treatment[a] on insect development in grain after the treatment material is mechanically removed from the grain.

CONCENTRATION (p.p.m.)	PER CENT KERNELS DAMAGED AFTER[b]				
	2 Mo.	5 Mo.	6 Mo.	9 Mo.	12 Mo.
10	2.2	1.0	1.0	1.5	2.5
Check	1.5	1.2	1.0	5.5	22.7

[a] Treatment consisted of a 5% lindane-sawdust mixture at the rate of 10 p.p.m. in the grain mass for 3 months before removal. The check grain was kept free of insects during this time by a series of fumigations. Initial damage of the grain in test was 1%.

[b] Resulting damage caused by the rice weevil.

containers was used to fill ½-gallon fiber cartons. Fifty adult rice weevils were introduced into each replicate and a 30-mesh screen secured over the tops. These cartons were stored in the grain storage room. Treatments were replicated four times. After 5 months the small-mesh screens were removed and replaced with ¼-inch screens. This permitted free movement of insects in and out of the containers. Five examinations were made during a 12 months' storage period (table 5).

Effect of the treatment on the immature stages of the rice weevil and the Angoumois grain moth.—Fresh shelled and fumigated corn was exposed to rice weevil and Angoumois grain moth adults for oviposition. After a 10-day exposure the grain was screened to remove all adult insects and transferred to ½-gallon fiber cartons with 30-mesh screen on the tops. Part of these containers were treated with 10 p.p.m. and 1 p.p.m. lindane from a 5% lindane-sawdust preparation. Each treatment was replicated four times. Examination was made after 2 months to determine the percentage of kernels which contained emergence holes by the species concerned (table 6).

Comparative effectiveness between equivalent amounts of lindane derived from 5% lindane-impregnated sawdust and 1% lindane dust.—A small-scale laboratory test, utilizing ½-gallon fiber cartons equipped with ¼-inch mesh screen lids and containing 3 pounds of shelled corn each, was conducted to determine this point. The lindane in each treatment was used at the rate of 4 p.p.m. For comparison, a DDT treatment at the rate of 7 p.p.m. derived from 5% dust was included in this test. The grain under observation in this test was stored in the grain storage room for 10 months. Treatments were replicated four times. The grain was examined for damage three times during the course of the experiment (table 7).

Crib tests in cooperation with growers.—A total of 17

Table 6.—Effect of lindane-sawdust treatment on the immature stages of the rice weevil and the Angoumois grain moth.

CONCENTRATION (p.p.m.)	PER CENT KERNELS WITH EMERGENCE HOLES AFTER TWO MONTHS	
	Rice Weevil	Angoumois Grain Moth
10	5*	70
1	(not tested)	81
Check	24.5	95
L.S.D. at 5% level		22

Table 7.—Relative effectiveness of two formulations of lindane and DDT against stored corn insects.

CONCENTRATION (p.p.m.)	PER CENT KERNELS DAMAGED AFTER[a]		
	3½ Mo.	7 Mo.	10 Mo.
4—lindane (from 1% dust)	1.0	1.0	1.5
4—lindane (from 5% dust impregnation)	1.2	1.5	2.5
7—DDT (from 5% dust)	1.2	1.5	1.0
Check	4.2	13.7	58.0
L.S.D. at 5%	3.0	8.1	17.0
at 1%	4.4	11.8	24.6

[a] The initial damage of this corn was 2%.

farm-type cribs were under test during the 1954–1955 storage season. These cribs represented the average type found in Louisiana. They were located in the southern half of the state in the areas most heavily damaged by stored grain insects. Since these tests were in cooperation with farmers, it was not possible to arrange for check cribs or check lots of corn in every case. These check lots represented from 10 to 100 bushels of corn stored by the farmer in a building separate from, but close to, his main crib. Treated cribs contained from 20 to 600 bushels of corn. Some of the cribs were lined with 15-pound builders' felt on the floor and the walls as high as the corn was piled. Most of these bins used in tests represented the typical open-type farm crib found in Louisiana. The treatments were made on a 1-foot rise in the corn level as the cribs were filled. The cribs were filled in September and October. The corn varieties represented were La. 521, Dixie 18, and Funk's G-737. The initial infestation was determined for each crib by the farmer cooperator removing at random approximately 20 ears from each load as he filled his crib. These ears were shelled and the average damage was determined from these samples by individual kernel examination. Crib examinations were made after 6, 8, 9, 10, 11 and 12 months' storage by removing at random 20 ears from the bottom to the top of each crib. These ears were shelled and the percentage of kernels damaged by insects in storage determined (table 8).

The technique used to determine kernel damage was as follows: After the ears were obtained they were shelled and the grain was thoroughly mixed. A subsample consisting of 1 quart was dipped up, and 1,000 kernels in lots of 100 were individually examined for emergence holes, visible larval channels, and damaged areas caused by stored grain insects. Five hundred kernels per treatment replicate were examined in the small-scale laboratory tests. These were removed at random from the container after the entire contents were thoroughly mixed. Samples from the large-scale shelled corn test were obtained with a grain probe.

RESULTS.—Table 1 shows the comparative value of different dosage levels of lindane derived from 5% lindane-sawdust formulation in the protection of shelled corn from damage during 13 months' storage in a small-scale laboratory test. A dosage of 0.5 p.p.m. gave complete protection in this test for the entire storage period. A dosage of 0.1 p.p.m. was adequate for a period of only 10

Table 8.—Effect of lindane at 10 p.p.m., derived from 5% impregnated sawdust, in protecting farm stored corn. 1954–1955.

Crib No.[a]	Category	Bushels and Variety	Per Cent Initial Damage	Per Cent Kernels Damaged After (Months)					
				6	8	9	10	11	12
1	Treated	300—Dixie 18	6	5	7	3	5	5	11
	Check	Not available							
2	Treated	200—Dixie 18	6	6	4	6	4	10	13
	Check	10—Dixie 18	6	14	24	39	29	60	42
3	Treated	250—La. 521	8	11	11	8	—	7	9
	Check	Not available							
4	Treated	300—La. 521	2	1	4	3	—	6	3
	Check	10—La. 521	2	4	10	20	—	10	38
5[b]	Treated	350—F-737	2	2	4	4	4	2	1
	Check	20—F-737	2	10	16	26	36	51	34
6	Treated	150—F-737	8	9	9	—	7	9	—
	Check	100—F-737	8	16	—	—	30	26	—
7	Treated	600—F-737	2	3	2	4	2	2	3
	Check	Not available							
8	Treated	300—F-737	8	15	8	—	10	10	8
	Check	Not available							
9[c]	Treated	400—mixed	2	1	2	—	1	1	1
	Check	10—mixed	2	7	7	—	41	62	60
10	Treated	20—Dixie 18	1	1	1	1	2	1	1
	Check	300—Dixie 18	1	9	8	26	31	31	45
11	Treated	20—Dixie 18	1	1	1	1	1	1	1
	Check	Same as No. 10							
12	Treated	20—Dixie 18	1	1	1	4	1	2	1
	Check	Same as No. 10							
13	Treated	20—Dixie 18	1	1	1	1	1	1	2
	Check	Same as No. 10							
14[c]	Treated	33—Dixie 18	1	2	3	1	1	2	1
	Check	9—Dixie 18	3	—	—	—	—	—	99
15[c]	Treated	33—Dixie 18	1	2	2	5	2	2	2
	Check	Same as No. 14							
16[c]	Treated	33—Dixie 18	8	8	6	8	13	8	8
	Check	Same as No. 14							

[a] All corn was snapped except as otherwise indicated.
[b] Shelled corn.
[c] Shucked corn.

months. Five-tenths p.p.m. in another laboratory test on shelled corn did not offer complete protection over 12 months' storage, but a dosage of 4 p.p.m. was completely effective (table 2).

The data presented in table 3 reveal the excellent protective quality lindane offers to snapped corn at dosages as low as 1 p.p.m. The tightness of bin construction had little, if any, effect on control of stored grain insects in snapped corn. The insect of importance in this test was the rice weevil. The contrast in protection as well as the insect concerned and the effect of tight versus ventilated bins is shown by comparing tables 3 and 4. The results presented in table 4 were from a test conducted with shucked corn. Dosages up to 4 p.p.m. were inadequate. The Angoumois grain moth was responsible for the damage in this test. Four p.p.m. protected the bottom half of the grain sample in tight storage but failed to effect protection in ventilated storage.

Shelled corn treated with lindane at 10 p.p.m. for 3 months, then separated from the treatment remained in perfect condition over a period of 12 months (table 5). This indicates that either the kernel absorbs the toxic principle or the vapors are sublimed on the grain.

Table 6 is presented to show the effect of the treatment on the immature stages of the rice weevil and the Angoumois grain moth. It was observed in the early stage of this work that immature development of the Angoumois grain moth and the rice weevil was little affected at dosages of 5 p.p.m. and less. Rice weevil larvae in ears from treated bins were observed to continue apparent normal development and to make their emergence holes. However, only a small percentage of the newly developed adults emerged. Those that did emerge from the grain were found to die shortly thereafter. The test reported in table 6 was designed to determine the exact effect lindane at 10 and 1 p.p.m. had on the larvae of the Angoumois grain moth, and the effect of 10 p.p.m. on the larval stage of the rice weevil. At 10 p.p.m. both species were adversely affected. One p.p.m. had no effect on the Angoumois grain moth.

Equivalent amounts of lindane derived from 1% dust and 5% sawdust impregnation were equally effective in protecting corn from damage for 10 months. DDT at 7 p.p.m. from 5% dust was as effective as the lindane treatments (table 7).

In tests conducted with farmers using corn produced and stored on the farm in typical open-type crib structures normally found in Louisiana, the lindane treatments were completely effective in each case in maintaining the corn in the same condition after 12 months' storage as when it was stored (table 8). These tests were conducted in the southern half of the State, which represents the area most heavily damaged by stored grain insects. The untreated lots of corn for these practical farm tests ranged from 34 to 99% damaged after 12 months. These tests were conducted on snapped, shucked, and shelled corn in papered and unpapered cribs or bins, at 10 p.p.m. lindane derived from 5% lindane-sawdust mixture. The first live weevil observed in any of these cribs was in early September, after 11 months' storage.

The effect of the lindane-sawdust treatment on other species of stored grain insects has not been determined. Observations made in conjunction with the experiments described above indicate that the confused flour beetle is affected very little, if any.

Chemical analysis of treated grain is in progress at this time.

SUMMARY.—Results of experiments in which lindane-impregnated sawdust was used as a protectant for stored corn, show that lindane at dosages as low as 1 p.p.m. was effective in protecting snapped corn for 12 months. Dosages up to 4 p.p.m. were inadequate to protect shucked corn. Ten p.p.m. gave complete protection to shucked corn, the difference between the two being that of insect species responsible for the damage. Lindane is apparently many times more toxic to the rice weevil than it is to the Angoumois grain moth. Tightness of construction of the bin appears to be an important factor though not a prerequisite for control. Shelled corn treated at 0.5 p.p.m. was found in one test to remain in perfect condition for 13 months. In another test this dosage rate failed to effect complete control. A treatment of 0.1 p.p.m. was effective for 10 months. Shelled corn is apparently protected at lower dosages than snapped or shucked corn. Grain previously treated with lindane, then separated from the treatment was found to exhibit either toxic or repellent qualities to stored grain insects for 12 months.

The immature stages of the rice weevil and the Angou-

mois grain moth are not materially affected by low dosages of lindane.

Equivalent amounts of lindane from a sawdust-impregnation and a dust formulation were equally effective in protecting corn.

In practical tests conducted with farmers in corn stored in open-type cribs and treated with lindane at 10 p.p.m., the corn remained in essentially the same condition after 12 months as when it was stored.

Since a tolerance for lindane on grain has not been established, this treatment cannot be recommended until the lindane residues resulting from the treatment have been determined to be safe.

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Effect of Sunlight and Other Factors on the Toxicity of Certain Insecticides[1]

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The influence of climatic factors on the effectiveness of insecticides applied to cotton plants for the control of insects has been under investigation at this Station since 1948. Results of the laboratory work conducted during the period 1948 to 1953 have been reported by Gaines & Dean (1949, 1950), Gaines & Mistric (1951, 1952), and Mistric & Gaines (1953, 1954). The results of these studies have shown that climatic factors such as rain, dew, humidity, temperature, sunlight, and wind are important forces which affect insecticidal toxicity.

Little laboratory work has been undertaken to determine the possibility of lengthening the period of residual effectiveness of insecticides by increasing the dosages of the materials above the recommended levels. On the basis of field investigations, approximately one-half of the workers report that increased dosages of insecticides have resulted in lengthening the period of residual effectiveness of materials while the remaining workers report negative results. The accumulative effect of repeated applications of insecticides, which could be measured by residual toxicity to certain pests, has been voiced as a distinct possibility by some investigators.

The objectives of this study were: (1) to determine the effect of dosage levels on the residual toxicities of insecticides, (2) to determine if residues toxic to insects tend to accumulate on plants receiving repeated insecticidal applications, (3) to obtain information regarding the independent effects of sunlight and rainfall on the toxicities of certain insecticides, and (4) to determine the residual effectiveness of certain promising new insecticides. The experiments reported herein were conducted in the laboratory at College Station, Texas during 1954.

MATERIALS AND METHODS.—Adult boll weevils, *Anthonomus grandis* Boh., and fourth-instar larvae of the cotton leafworm, *Alabama argillacea* (Hbn.), were field-collected. Toxaphene, BHC, dieldrin, heptachlor, aldrin, endrin, parathion, Strobane, and calcium arsenate were used in these tests. Materials included for the first time were Chlorthion and Bayer 17147.

All of the materials, except calcium arsenate dust, were applied as emulsion sprays to mature cotton plants growing in pots. The sprays were made by diluting emulsifiable concentrates with water and were applied at a pressure of 60 p.s.i. through two hollow cone nozzles at the rate of 5 gallons per acre. The plants were rotated through the spray at 5 m.p.h. by means of a turn-table device. The calcium arsenate dust was applied by means of a sharp air blast from the top of a tower 18 inches in diameter by 44 inches in height. The dosages are shown as pounds of active ingredients per acre. Screened cages were used to confine the insects to treated plants.

Insect survival records were made during a 3-day period following release of insects on treated plants. These records were based on the number of dead individuals and death was defined as cessation of all visible movement in the specimens. Untreated checks were used in each experiment. Percentage control was calculated by Abbott's formula.

Most experiments were replicated four times on each of four or six dates. Fifteen weevils or 10 worms were used per replicate. The percentage control in each boll weevil experiment is based upon the use of at least 240 weevils with each insecticidal treatment under each set of indicated test conditions. The data on the cotton leafworm are based upon the use of at least 160 leafworms.

Some of the treated cotton plants were exposed to various climatic conditions outside of the laboratory and inside of the laboratory prior to the release of insects on all plants in the laboratory. Treated plants exposed to outdoor weather conditions (with the exception of experiments 1 and 5) did not receive any natural rainfall. In all tests the pests were released on treated plants immediately (30 minutes) after treatment, and in certain tests the pests were also released 24 or 48 hours following the treatment of plants. Random samples of the same population of insects (with the exception of experiment 5) were released on treated plants in all of the various categories

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Table 1.—Toxicity test for boll weevil control with insecticidal sprays. College Station, Texas, 1954.[a]

Treatment	Dose[b]	Per Cent Control		Per Cent Reduction in Toxicity Due to 24-Hour Delay, Rain[c]
		Immediate Release	24-Hour Delay Rain[c]	
Toxaphene	2.0	57	38	33
BHC	0.33	83	6	93
Dieldrin	0.33	100	45	55
Heptachlor	0.33	97	18	81
Aldrin	0.33	100	0	100

[a] Laboratory conditions during test period—temperature: max. 88°, min. 81° F.; relative humidity: max. 99, min. 63%.
[b] Pounds active ingredient per acre applied in July.
[c] Outdoor conditions during 24-hour exposure period—temperature: max. 95°, min. 71° F.; relative humidity: max. 99, min. 32%; 0.87 inch rainfall; 2.8 m.p.h. wind velocity; no dew; and cloudy atmosphere.

on the same date. This was accomplished by treating the plants involving 24- or 48-hour delay periods 1 or 2 days before the date of release of insects and beginning of each test period.

RESULTS.—*Experiment 1.*—This test was conducted to determine the effect of natural rainfall on the residual toxicities of toxaphene, BHC, dieldrin, heptachlor, and aldrin when used to control the boll weevil (table 1). Aldrin, BHC, and heptachlor were ineffective following a 24-hour exposure of treated plants to 0.87 inch of rainfall. The toxicity of toxaphene was reduced less than that of dieldrin by the rainy weather. Appreciable control was obtained with toxaphene or dieldrin following the exposure of treated plants to rainy conditions, although the toxicities of these materials were reduced.

Experiment 2.—This test was conducted to determine the effect of sunlight on the toxicities of toxaphene, BHC, dieldrin, heptachlor, and aldrin when used to control the boll weevil (table 2). The toxicities of BHC, heptachlor, and aldrin were greatly reduced following a 24-hour exposure of treated plants to high temperature under shaded conditions outside the laboratory. Similarly treated plants exposed to sunlight under the same conditions showed only a slight additional loss in toxicity. At a high temperature, sunlight was not an important factor in reducing the effectiveness of these materials. High temperature alone caused practically a total loss in insecticidal toxicity. Toxaphene and dieldrin retained considerable toxicity following the exposure of treated plants to high temperature and sunlight. Plants treated with toxaphene showed no loss in toxicity due to the effect of sunlight. Sunlight was an important factor in reducing the toxicity of dieldrin.

Table 2.—Toxicity test for boll weevil control with insecticidal sprays. College Station, Texas. 1954.[a]

Treatment	Dose[b]	Per Cent Control			Per Cent Reduction in Toxicity in 24 Hours[c]	
		Immediate Release	24-Hour Delay[c]			
			Shade	Sunlight	Shade	Sunlight
Toxaphene	2.0	53	41	45	22	15
BHC	0.33	48	8	6	84	87
Dieldrin	0.33	88	62	42	30	52
Heptachlor	0.33	54	8	7	85	87
Aldrin	0.33	60	9	8	85	87

[a] Laboratory conditions during test period—temperature: max. 94°, min. 82° F.; relative humidity: max. 84, min. 41%.
[b] Pounds active ingredient per acre applied in August and September.
[c] Outdoor conditions during 24-hour exposure period—temperature: max. 99°, min. 72° F.; relative humidity: max. 99, min. 22%; 4.3 m.p.h. wind velocity; light dew; clear atmosphere; and full sunlight or shade.

Table 3.—Toxicity test for boll weevil control with insecticidal sprays. College Station, Texas. 1954.[a]

Treatment	Dose[b]	Per Cent Control	
		Immediate Release	48-Hour Delay[c]
Toxaphene	2.0	56	38
Toxaphene	3.0	60	37
BHC	0.33	71	2
BHC	0.45	78	4

[a] Laboratory conditions during test period—temperature: max. 92°, min. 81° F.; relative humidity: max. 88, min. 49%.
[b] Pounds active ingredient per acre applied in July, August and September.
[c] Outdoor conditions during 48-hour exposure period—temperature: max. 100°, min. 72° F.; relative humidity: max. 99, min. 26%; 2.9 m.p.h. wind velocity; medium dew; and clear atmosphere.

Experiments 3 and 4.—These tests were conducted to determine the effect of dosage levels on the initial and residual toxicities of toxaphene and BHC in experiment 3 (table 3) and endrin and heptachlor in experiment 4 (table 4) when used to control the boll weevil. Each insecticide was applied at a dosage which would result in approximately 75% control, and at a dosage 1½ times as great. Increased dosages of the materials resulted in only slightly increased initial insect mortality. This small increase in initial toxicity was not sufficient to justify the use of increased dosages. Increased dosages of these materials did not increase residual effectiveness during 48-hour exposures of treated plants to natural climatic conditions. BHC and heptachlor applied at either dosage level were virtually ineffective 48 hours after application; toxaphene retained considerable toxicity while endrin retained a small degree of toxicity during the same period.

Experiment 5.—This test was conducted to determine the initial and residual effectiveness of multiple applications of toxaphene and BHC to the boll weevil (table 5). The primary objective of this experiment was to determine if residues toxic to the boll weevil tend to accumulate on cotton plants receiving repeated applications of these insecticides. The data indicate that multiple applications of toxaphene or BHC did not result in accumulated toxic

Table 4.—Toxicity test for boll weevil control with insecticidal sprays. College Station, Texas. 1954.[a]

Treatment	Dose[b]	Per Cent Control	
		Immediate Release	48-Hour Delay[c]
Endrin	0.12	87	27
Endrin	.18	90	28
Heptachlor	.36	63	6
Heptachlor	.54	67	6

[a] Laboratory conditions during test period—temperature: max. 90°, min. 81° F.; relative humidity: max. 87, min. 49%.
[b] Pounds active ingredient per acre applied in September and October.
[c] Outdoor conditions during 48-hour exposure period—temperature: max. 95°, min. 67° F.; relative humidity: max. 92, min. 23%; 3.8 m.p.h. wind velocity; light dew; and partly cloudy atmosphere.

Table 5.—Toxicity test for boll weevil control with insecticidal sprays. College Station, Texas. 1954.[a]

Treatment	Dose[b]	Per Cent Control: Immediate Release Following Indicated No. of Applications[d] 1	2	4	48-Hour Delay[c] Following Indicated No. of Applications[d] 1	2	3
Toxaphene	2.67	42	43	41	31	38	38
BHC	0.37	75	74	63	17	22	21

[a] Laboratory conditions during test period—temperature: max. 89°, min. 79° F.; relative humidity: max. 74, min. 42%.
[b] Pounds active ingredient per acre applied in August, September and October.
[c] Outdoor conditions during 48-hour exposure period—temperature: max. 89°, min. 62° F.; relative humidity: max. 90, min. 20%; 2.8 m.p.h. wind velocity; medium dew; and clear atmosphere.
[d] Number of applications applied at 5-day intervals; the last application made immediately, or 48 hours, before the release of insects; all treated plants exposed to outdoor weather conditions throughout the application periods.

residues which could be measured in terms of either initial or residual toxicity to the boll weevil. A minor trend existed which indicated that repeated applications of these materials decreased the initial toxicity and increased the residual toxicity of subsequent applications; however, these small differences were of little significance.

Experiment 6.—This test was conducted to compare the initial and residual effectiveness of three experimental materials to that of toxaphene when used to control the boll weevil (table 6). Strobane compared very favorably with toxaphene from the standpoints of initial control and residual effectiveness following laboratory exposures of treated plants. Strobane was somewhat more stable than toxaphene under natural weather conditions. Under laboratory and outdoor exposures, the toxicity of Chlorthion was reduced slightly more than that of toxaphene. Bayer 17147 at a low dosage was found to possess outstanding residual properties under conditions of laboratory and outdoor exposures. The residual effectiveness of this insecticide was far superior to that of the other materials tested, indicating the urgent need for additional research with it.

Table 6.—Toxicity test for boll weevil control with insecticidal sprays. College Station, Texas. 1954.[a]

Treatment	Dose[b]	Per Cent Control: Immediate Release	24-Hour Delay: Indoor[a]	24-Hour Delay: Outdoor[c]	Per Cent Reduction in Toxicity in 24 Hours: Indoor[a]	Per Cent Reduction in Toxicity in 24 Hours: Outdoor[c]
Toxaphene	3.0	54	41	25	24	54
Strobane	3.0	48	36	32	25	34
Chlorthion	0.23	92	57	38	38	58
Bayer 17147	0.12	87	83	82	4	5

[a] Laboratory conditions during test period—temperature: max. 88°, min. 78° F.; relative humidity: max. 63, min. 35%.
[b] Pounds active ingredient per acre applied in September and October.
[c] Outdoor conditions during 24-hour exposure period—temperature: max. 91°, min. 63° F.; relative humidity: max. 95, min. 28%; 3.9 m.p.h. wind velocity; heavy dew; and partly cloudy atmosphere.

Experiment 7.—This test was conducted to determine the effect of ½ inch of simulated rainfall on the toxicities of toxaphene, endrin, parathion, and calcium arsenate when used to control the cotton leafworm (table 7). The toxicities of toxaphene and calcium arsenate were reduced to a lesser extent than those of endrin and parathion during a 24-hour exposure of treated plants to natural climatic conditions in the absence of rainfall. However, all of these insecticides showed good residual properties under these conditions. A slight but consistent trend with all materials indicated that rain occurring immediately after treatment was slightly more detrimental to insecticidal effectiveness than rain occurring 24 hours after treatment. Rain slightly reduced the residual effectiveness of toxaphene and parathion, appreciably reduced the effectiveness of endrin, and greatly reduced the effectiveness of calcium arsenate.

SUMMARY.—Laboratory experiments were conducted at College Station, Texas during 1954 to determine the effects of climatic factors, insecticidal dosage levels, and multiple insecticidal applications on the effectiveness of certain insecticides used to control the boll weevil, *Anthonomus grandis* Boh., and the cotton leafworm, *Alabama argillacea* (Hbn.). Results of this study were as follows:

(1) BHC, heptachlor, and aldrin were completely ineffective in controlling the boll weevil following a 24-hour exposure of treated plants to 0.87 inch of natural rainfall. Appreciable control was obtained with toxaphene and dieldrin under these conditions.

(2) A 24-hour exposure of treated plants in outdoor shade at high temperature greatly reduced the residual effectiveness of BHC, heptachlor, and aldrin when used to control the boll weevil. High temperature alone caused practically a total loss in insecticidal toxicity, hence sunlight was not an important factor in reducing the effective-

Table 7.—Toxicity test for cotton leafworm control with insecticidal sprays. College Station, Texas. 1954.[a]

Treatment	Dose[b]	Per Cent Control: Immediate Release	24-Hour Delay[c]: No Rain	½ Inch Simulated Rain: 30 Minutes After Treatment	½ Inch Simulated Rain: 24 Hours After Treatment	Per Cent Reduction in Toxicity Due to: 24-Hour Delay, No Rain	Rain 30 Min. After Treatment	Rain 24 Hours After Treatment
Toxaphene	0.88	70	61	50	51	12	16	14
Endrin	0.09	78	61	32	38	21	37	30
Parathion	0.04	96	76	66	73	21	10	3
Calcium arsenate	6.75	82	74	9	25	10	80	60

[a] Laboratory conditions during test period—temperature: max. 89°, min. 78° F.; relative humidity: max. 62, min. 34%.
[b] Pounds active ingredient per acre applied in September, October and November.
[c] Outdoor conditions during 24-hour exposure period—temperature: max. 90°, min. 64° F.; relative humidity: max. 98, min. 21%; 4.1 m.p.h. wind velocity; light dew; and clear atmosphere.

ness of these materials. Toxaphene and dieldrin retained considerable toxicity in outdoor sunlight at high temperature. Sunlight did not affect the toxicity of toxaphene, while it was an important factor in reducing the toxicity of dieldrin.

(3) Increased dosages of toxaphene, BHC, heptachlor, and endrin slightly increased the initial control of the boll weevil, but not sufficiently to justify the use of the increased dosages. When plants treated at either dosage were exposed for 48 hours to outdoor weather conditions, BHC and heptachlor were virtually ineffective while endrin retained a small degree of toxicity. Toxaphene retained considerable toxicity under these conditions.

(4) Repeated applications of either toxaphene or BHC at 5-day intervals did not result in accumulated toxic residues which could be measured in terms of either initial or residual control of the boll weevil.

(5) When treated plants were exposed for 24 hours indoors or outdoors, the toxicity of Chlorthion to the boll weevil was reduced slightly more than that of toxaphene. Strobane compared very favorably with toxaphene in both initial and residual control. The residual effectiveness of Bayer 17147 at a low dosage was far superior to that of toxaphene.

(6) The effectiveness of parathion or endrin in controlling the cotton leafworm was reduced to a greater extent than that of toxaphene or calcium arsenate during a 24-hour exposure of treated plants to natural climatic conditions in the absence of rainfall. However, all of these materials exhibited good residual properties under these conditions. One-half inch of simulated rainfall applied immediately after insecticide applications was slightly more detrimental to effectiveness than rain occurring 24 hours after treatment. Rain slightly reduced the toxicity of toxaphene and parathion, appreciably reduced the toxicity of endrin, and greatly reduced the toxicity of calcium arsenate.

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Effect of Climatic Conditions on the Chemical Control of Certain Sucking Pests of Cotton[1]

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It has been rather widely reported that extremely variable degrees of control of spider mites and the cotton aphid have been obtained under field conditions during recent years. The first substantial amount of work on the effect of organic insecticides on predators of these pests, the susceptibility of different species of spider mites to newer acaricides, and the resistance of these pests to chemicals were reported by Newsom & Smith (1949), Mistric & Rainwater (1952), and Ivy & Scales (1954).

Little information, other than field observations, has been accumulated by investigators on the effect of climatic conditions on the chemical control of these pests. On the basis of field observations it has been generally accepted that in order to obtain adequate control of these pests applications of chemicals should be made so as to provide complete coverage of plants when the air is nearly calm and the temperature is not excessively low.

The objective of this study was to obtain additional information regarding the effect of weather conditions on the toxicity of certain materials currently used for the control of spider mites and the cotton aphid. The experiments reported herein were conducted in the laboratory or greenhouse at College Station, Texas during 1954.

MATERIALS AND METHODS.—Parathion and Aramite were used in tests with the two-spotted spider mite, *Tetranychus telarius* (L.), and the desert spider mite, *Tetranychus desertorum* Banks. Parathion and BHC were used in tests with the cotton aphid, *Aphis gossypii* Glov. All of these pests were laboratory-reared from field populations collected locally. The aphid tests were begun with randomly mixed populations of immature and mature forms, including a small percentage of winged individuals. Survival records were made on all stages collectively. The spider mite tests were begun with populations averaging approximately 90% adults and 10% nymphs. Survival records were made on adult and nymphal stages collectively.

All of the materials were applied as emulsion sprays to seedling cotton plants (cotyledonous stage) grown in nutrient solution. The sprays were made by diluting emulsifiable concentrates with water, and were applied from the

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top of a spray tower through a solid cone nozzle at a pressure of 40 p.s.i. at the rate of 7.5 gallons per acre. Plants in an upright position at the bottom of the tower were rotated through the spray. The spray tower was 18 inches in diameter and 44 inches in height.

Following the insecticide application, each plant was placed in a small, narrow-necked bottle filled with nutrient solution. Absorbent cotton was used at the neck of the bottle to hold the plant in a stationary, upright position. Six plants were included under each test condition. These plants were selected at random from an entire group of plants receiving a particular treatment on a certain date.

Untreated pests were transferred to treated plants at various intervals after insecticide applications. The transfer of pests was accomplished by counting the number of pests on a section of an infested leaf and fastening the leaf section to a treated leaf with an insect pin. The leaf sections dried rapidly, forcing most of the pests to transfer to the treated leaves within 2 hours. An average of 30 pests was transferred to each treated seedling.

Untreated checks were used in each test. Percentage control was calculated according to Abbott's formula. Hygrothermographs were used to record the temperature and relative humidity. The dosages of insecticides are expressed as pounds of active ingredients per acre. Survival records were made daily during the 5-day period following the transfer of pests to treated plants. These records were based on the number of live individuals. Life was defined as the presence of any visible movement in the specimens when they were gently prodded with a blunt needle and viewed through a 10-power binocular scope.

In all tests designated as "immediate release," the pests were transferred to treated plants immediately (30 minutes) after treatment, while in other tests the pests were also released 24 or 72 hours following the treatment of plants. Random samples of the same population of pests were released on treated plants in all of the various categories on the same date. This was accomplished by treating the plants involving 24- and 72-hour delay periods 1 and 3 days before the date of release of pests and beginning of each 5-day test period.

Experiment 1 was conducted under three temperature conditions. The test at low temperature was conducted in the laboratory in a constant temperature and humidity cabinet. The test at medium temperature was conducted on a laboratory shelf protected from air currents and sunlight. These tests were conducted under fluorescent lights in order to maintain healthy seedling plants. The test at high temperature was conducted in a closed, shaded greenhouse. This experiment was begun on June 18 and duplicated on July 5. The percentage control shown under each experimental condition was based upon the use of 360 aphids.

In experiment 2 the test periods for each pest were as follows: cotton aphid, March 18 to May 12; desert spider mite, April 22 to June 26; and two-spotted spider mite, March 16 to July 24. The climatic conditions during these tests differed somewhat with each pest. Following spray applications and climatic exposures, the 5-day test period with the cotton aphid was conducted in a constant temperature and humidity cabinet, while the 5-day test periods with the spider mites were conducted on a laboratory shelf protected from air currents and sunlight. The tests with all pests were repeated on six dates. The percentage control shown under each experimental condition was based upon the use of 1,080 pests.

In experiment 2 one group of treated plants was placed on a laboratory shelf for 24 hours before pests were transferred to them. These plants were not exposed to any weather factors other than the prevailing temperature and humidity in the laboratory. This group of plants served as the basis for determining the effects of dew, rain, and wind when applied separately to other similarly-treated groups of plants. This group of plants also served to compare the control obtained at laboratory temperature with that obtained at a higher temperature in a shaded greenhouse. The effect of sunlight was determined by comparing the control obtained with treated plants exposed to greenhouse sunlight to that obtained with treated plants exposed to greenhouse shade at the same temperature. Temperature in the shade was increased to that in sunlight by the use of an uninsulated cover which dropped half-way down the sides. Probably the full effect of sunlight may not have been measured since it is estimated by several authorities that greenhouse glass may reduce the amount of ultraviolet radiation by approximately 75%. Dew was simulated on treated plants by applying a fine mist of water with a continuous sprayer to the point of run-off. Simulated rain was applied at the rate of $\frac{1}{2}$ inch by the use of a garden sprinkler and screen window arrangement. Dew or rain was applied 30 minutes after the spray applications. Wind was simulated by the use of a 16-inch oscillating electric fan. Treated plants were exposed to a 4.5 m.p.h. air movement for 24 hours.

RESULTS.—*Experiment 1.*—This test was conducted to determine the effect of temperature on the initial and residual toxicities of parathion and BHC to the cotton aphid (table 1). Since the aphids were not sprayed with the insecticides (only the plants were treated), higher dos-

Table 1.—Laboratory toxicity tests for cotton aphid control with insecticidal sprays. College Station, Texas. 1954.

Treatment	Maximum Temperature[c] (Degrees F.)	Cumulative Per Cent Control on Days Indicated Following Aphid Release					Average Daily Per Cent Control	Per Cent Reduction in Toxicity During Indicated Delay Periods
		1	2	3	4	5		
Immediate Release								
Parathion[a]	70	87	92	92	93	93	91	
	94	100	100	100	92	82	95	
	107	90	94	96	95	95	94	
BHC[b]	70	48	50	47	48	35	46	
	94	65	79	69	57	46	63	
	107	24	54	47	52	44	44	
1-Day Delay								
Parathion	70	18	31	24	35	38	29	68
	94	56	73	85	86	96	79	17
	107	34	55	60	57	55	52	45
BHC	70	4	0	0	0	0	1	98
	94	0	3	0	0	0	1	98
	107	10	12	13	8	7	10	77
3-Day Delay								
Parathion	70	13	16	17	17	8	14	85
	94	14	32	39	42	50	35	63
	107	18	27	34	34	37	30	68
BHC	70	7	6	8	0	0	4	91
	94	0	4	0	0	0	1	98
	107	4	6	10	6	6	6	86

[a] 0.25 pound active ingredient per acre.
[b] 1.2 pounds active ingredient per acre.
[c] Mean relative humidity at each temperature approximately 65%.

ages of insecticides were required for control under these conditions. In general, the results indicated that following applications of either parathion or BHC, the cotton aphid was most effectively controlled at a medium temperature. From the standpoint of residual toxicity, parathion was most effective in controlling the aphid at a medium temperature, and it was more effective at a high temperature than at a low temperature. From the standpoint of initial control, parathion was equally effective in controlling the aphid at low, medium and high temperatures. The greatest initial control of the aphid with BHC was obtained at a medium temperature, and this material was equally effective at low and high temperatures. BHC was entirely ineffective in controlling the aphid at all three temperatures 1 day after application. At medium and high temperatures parathion showed an appreciable residual toxicity up to 3 days after application.

Under all three temperature conditions the initial aphid control with BHC reached its maximum within 48 hours and declined thereafter. By taking into consideration the time required for aphids to die, it can be concluded that BHC was effective for only a few hours. This is in agreement with results of the 1- and 3-day delay tests. Since three times the maximum recommended dosage of BHC was used in this experiment to obtain a substantial degree of aphid control (because the insects were not sprayed directly and the effectiveness of this material is of short duration), rather complete coverage of aphids with BHC during the spray application appears to be of the utmost importance. Under field conditions such coverage is often not easily attainable when cotton leaves and buds are extremely crinkled and curled. Any aphid control obtained with a single application of BHC under field conditions is likely to be of short duration in certain instances provided other control factors (parasites, predators, disease, environmental conditions) are not acting simultaneously with the insecticide.

Experiment 2.—This experiment was conducted to determine the effect of dew, rain, wind, temperature, and sunlight on the toxicities of parathion and Aramite when used to control the desert spider mite and the two-spotted spider mite, and parathion and BHC when used to control the cotton aphid (table 2). In this experiment the use of BHC spray resulted in a more effective initial and residual control of the cotton aphid than it did in experiment 1. The climatic conditions which prevailed during the two experiments were not sufficiently different to account for this difference in insect control. Moreover, very similar initial and residual aphid control was obtained with parathion in both experiments. These data clearly in-

Table 2.—Laboratory toxicity tests for control of spider mites and cotton aphid with chemical sprays. College Station, Texas. 1954.

Test Condition[a]	Pest[b]	Average Daily Per Cent Control			Per Cent Reduction in Toxicity in 24 Hours Due to Specific Climatic Factors[f]		
		Parathion[c]	Aramite[d]	BHC[e]	Parathion	Aramite	BHC
Immediate release	Desert spider mite	81	83				
	Two-spotted spider mite	93	86				
	Cotton aphid	91		82			
24-Hour delay							
In laboratory[g]	Desert spider mite	55	17		32	80	
(Unexposed)	Two-spotted spider mite	68	41		27	52	
	Cotton aphid	67		46	26		44
In laboratory	Desert spider mite	51	20		5	0	
(Heavy dew)	Two-spotted spider mite	67	40		1	1	
	Cotton aphid	51		46	18		0
In laboratory	Desert spider mite	41	8		17	10	
(½ inch rain)	Two-spotted spider mite	48	30		21	13	
	Cotton aphid	46		38	23		10
In laboratory	Desert spider mite	43	15		15	2	
(4.5 m.p.h. wind)	Two-spotted spider mite	53	40		16	1	
	Cotton aphid	55		51	14		0
In greenhouse shade[h]	Desert spider mite	49	20		8	0	
(High temperature)	Two-spotted spider mite	61	31		7	12	
	Cotton aphid	57		37	11		11
In greenhouse sun	Desert spider mite	28	12		25	10	
(Sunlight)	Two-spotted spider mite	58	32		4	0	
	Cotton aphid	50		30	8		8

[a] Mean relative humidities throughout this experiment approximately 65%.
[b] Maximum temperature during 5-day test periods: desert spider mite, 89°; two-spotted spider mite, 95°; and cotton aphid, 70° F.
[c] Desert spider mite 0.09, two-spotted spider mite 1.0, and cotton aphid 0.24 pound active ingredient per acre, respectively.
[d] Desert spider mite 0.61, and two-spotted spider mite 1.5 pounds active ingredient per acre, respectively.
[e] Cotton aphid 1.0 pound active ingredient per acre.
[f] Indicated in parentheses under test conditions.
[g] Maximum temperature: desert spider mite 87°, two-spotted spider mite 92°, and cotton aphid 83° F.
[h] Maximum temperature: desert spider mite 115° two-spotted spider mite 113°, and cotton aphid 105° F.

dicate the extreme difficulty involved in predicting the precise climatic conditions under which BHC would be effective in controlling the cotton aphid.

The residual toxicity of BHC to the cotton aphid was less severely affected by the climatic factors studied than was parathion. The greatest loss in the residual effectiveness of BHC was probably due to the innate chemical and physical properties of this insecticide. This loss in insecticidal toxicity occurred somewhat independently of climatic influences, although it was accelerated by certain weather factors. The toxicity of BHC was reduced more than that of parathion during a 24-hour exposure of treated plants in the laboratory. Parathion showed residual properties equal or superior to BHC under all of the various climatic exposures to which residues were subjected. Each of the individual climatic factors studied reduced to some extent the residual effectiveness of parathion. Only rain, high temperature, and sunlight reduced the residual effectiveness of BHC. In no instance did any single climatic factor reduce the residual toxicities of these insecticides to the point of complete ineffectiveness.

Parathion exhibited approximately the same residual activity against each species of spider mite as it did against the cotton aphid. Aramite exhibited poor residual properties when used to control either species of spider mite. The toxicity of Aramite was greatly reduced during a 24-hour exposure of treated plants in the laboratory. The toxicity of Aramite was further reduced by the various climatic factors studied; however, the toxicity of this material was reduced by these factors to a lesser extent than parathion. Although there was little difference in the initial toxicity of Aramite (at the dosages used) to the two species of spider mites, this material was residually more effective in controlling the two-spotted spider mite than the desert spider mite.

Aramite has sometimes appeared to exhibit good residual toxicity in the control of these spider mites under field conditions. Daily laboratory records showed that Aramite reduced spider mite populations very slowly. Under laboratory conditions the control of spider mites, due to applications of Aramite, increased steadily from the first to the fifth day following treatment. Slow control can easily be confused with residual toxicity, although the two phenomena are entirely different.

SUMMARY.—Laboratory experiments were conducted at College Station, Texas during 1954 on the effect of weather conditions on the toxicity of parathion and Aramite when used to control the desert spider mite, *Tetranychus desertorum* Banks, and the two-spotted spider mite, *Tetranychus telarius* (L.), and parathion and BHC when used to control the cotton aphid, *Aphis gossypii* Glov. All materials were applied as emulsion sprays to cotton seedlings.

The cotton aphid was more effectively controlled with parathion or BHC at medium temperature than at either low or high temperatures. BHC was residually ineffective at all three temperatures 1 day after application. At medium and high temperatures, parathion showed appreciable residual toxicity up to 3 days after application.

Erratic control of the cotton aphid was obtained with BHC under very similar climatic conditions. Consistent control was obtained with parathion under the same conditions. The data indicate the extreme difficulty involved in predicting with any reasonable degree of accuracy the climatic conditions under which BHC would be most effective.

The greatest decrease in the residual toxicity of BHC to the cotton aphid occurred somewhat independently of climatic influences, although the loss in toxicity was accelerated by certain weather factors. Parathion showed residual properties equal or superior to BHC under all of the various climatic exposures to which residues were subjected. Each of the individual climatic factors studied (dew, rain, wind, high temperature, sunlight) reduced to some extent the residual effectiveness of parathion.

Parathion exhibited approximately the same residual activity against each species of spider mite as it did against the cotton aphid. The greatest decrease in the residual toxicity of Aramite to these spider mites occurred somewhat independently of climatic influences, although the loss in toxicity was accelerated by certain weather factors. Parathion showed residual properties superior to Aramite under all of the various climatic exposures to which residues were subjected. Aramite was residually more effective in controlling the two-spotted spider mite than the desert spider mite. Aramite reduced spider mite populations very slowly—a factor easily confused with residual toxicity.

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BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

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Control of the Brown Cotton Leafworm, *Acontia dacia* Druce[1]

E. P. Lloyd and Dial F. Martin,[2] *Texas Agricultural Experiment Station, College Station*

Druce (1900) described the brown cotton leafworm, *Acontia dacia*, from specimens collected in Vera Cruz, Mexico, and Panama. During July, 1953 the larvae were reported by C. R. Parencia attacking cotton in Austin, Robertson, and Wharton counties in Texas. Martin & Mistric (1954) reported that the larvae caused considerable damage to several thousand acres of cotton in Brazos, Robertson, Wharton, Burleson, and Matagorda counties of Texas during 1954. In the Cooperative Economic Insect Report (1954) Burns and Newsom reported larval infestations in Natchitoches and Red River parishes in Louisiana during October.

Preliminary laboratory and field tests conducted during 1954 by Martin & Mistric (1954) indicated that 0.25 pound of parathion or 0.33 pound of endrin applied per acre was effective for the control of this pest. A heavy infestation of the brown cotton leafworm developed on cotton early in 1955 offering an opportunity to conduct laboratory and field tests to determine the effectiveness of a number of compounds in controlling the pest.

Materials.—The following insecticides applied as sprays were used in the experiments: parathion, Bayer 17147, endrin, malathion, Hercules AC-528 (2,3-dioxanedithiol *S,S*-bis- (*O,O*-diethylphosphorodithioate)), Chlorthion, Diazinon, Pyrazoxon, toxaphene, aldrin, BHC and heptachlor.

Methods.—*Laboratory Tests.*—The relative toxicity of several compounds to fourth instar larvae was determined by spraying cotton plants grown in 1-gallon cans. Measured amounts of emulsion were applied to the plants using two hollow cone nozzles calibrated to deliver 5 gallons of spray per acre at 60 pounds pressure per square inch. Plants were rotated through the spray at 4.5 miles per hour on a circular rotary device equipped to carry four potted cotton plants as described by Gaines & Dean (1950). Treated plants were allowed to dry and caged immmediately.

Fourth instar, laboratory-reared larvae were used in these tests. Eggs were laid by fertile female moths which were caged in lantern globes (approximately 8.5 inches in diameter and 11 inches high) resting on moist paper toweling. Eggs were attached to the sides of the lantern globes and to the moist paper toweling. Lantern globes containing the eggs were placed over potted cotton plants where the eggs hatched. Larvae in each globe were approximately the same age since the moths lay eggs only at night and were transferred to other lantern globes each day. The larvae were allowed to feed on cotton plants for 2 to 3 days. Second instar larvae were transferred to a large growing cotton plant caged in a 2×2×2 feet cage where the larvae were allowed to feed until they reached the fourth instar.

About 10 fourth instar larvae were released on each treated plant. Checks of untreated plants were included with each series of tests. Mortality was recorded at 24-hour intervals for 5 days. Each dosage-mortality curve was established by treating four plants at each dosage with an average of four different dosages of each insecticide. All mortality percentages were calculated by Abbott's formula. Dosage-mortality curves were calculated by the method of Bliss (1938).

Field Experiments.—Insecticides recommended for early-season control of insects on seedling cotton in Texas were tested against the brown cotton leafworm in experiment 1. Three phosphorus compounds, parathion, Diazinon, and malathion, were each tested at two different dosages on seedling cotton in experiment 2. The cotton in both of these experiments was between the 4- and 8-leaf stage. The average infestation of brown cotton leafworms was 25 and 21 larvae per 50 linear feet of row in experiments 1 and 2, respectively. This was the first record of this pest attacking seedling cotton in Texas.

Experiment 3 was conducted to determine the effectiveness in the field of several compounds which had proven most effective in laboratory tests. This experiment was conducted late in the season on mature cotton where the larval infestation averaged 122 larvae per 10 linear feet of row. In addition to this heavy larval infestation, a large moth population continued to lay eggs causing a reinfestation of all plots following treatment.

The insecticide treatments and an untreated check were randomized and replicated four times in all experiments. Plots 1/12 acre in size (12 rows wide and 91 feet long) were used in experiments 1 and 2. Infestation records were made on the same staked 50 linear feet of row in each plot in these two experiments. In experiment 3, plots 1/40 acre in size (6 rows wide and 54.5 feet long) were used. All infestation records were made on the same staked 10 linear feet of row in each plot.

Insecticides in all field experiments were applied with a 3-row power sprayer. A total of 2.5 gallons of spray per acre using one hollow cone nozzle per row was used in experiments 1 and 2 and 7.5 gallons of total spray per acre using three hollow cone nozzles per row were applied in experiment 3. Experiment 1 was treated April 30; experiment 2, May 4; and experiment 3, August 31.

The initial infestation record was made 1 day prior to treatment in all experiments and additional records were made on days following treatment as indicated in table 2. An analysis of variance of the percentage of living larvae was made for all experiments.

Results.—*Laboratory Tests.*—The dosage-mortality curves of the several compounds tested in the laboratory for the control of the brown cotton leafworm are shown in figure 1. The median lethal dose (LD^{50}) and the regression coefficient indicating the slope of the dosage-mortality curves for each compound are shown in table 1. Several of the phosphorus compounds and endrin, a chlorinated hydrocarbon, showed a high degree of toxicity to this insect. Parathion was the most toxic compound

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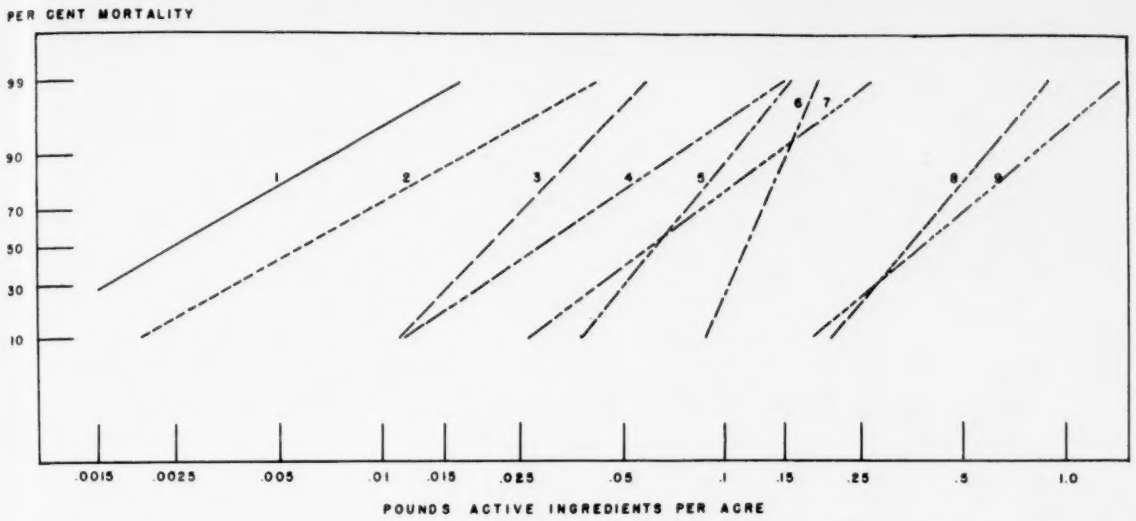

FIG. 1.—Dosage-mortality curves for the toxicity of the following insecticides to the 4th instar larvae of the brown cotton leafworm: (1) parathion, (2) Bayer 17174, (3) endrin, (4) malathion, (5) Hercules AC-528, (6) Chlorthion, (7) Diazinon, (8) toxaphene, and (9) Pyrazoxon.

Table 1.—Dosage-mortality relationship of several compounds against the fourth instar larvae of the brown cotton leafworm evaluated 5 days after application. (Laboratory Tests.)

COMPOUND	POUNDS OF ACTIVE INGREDIENTS PER ACRE REQUIRED TO GIVE 50% MORTALITY	SLOPE OF LINE
Parathion	0.0025 ± .0005	2.73
Bayer 17147	.0057 ± .0013	2.63
Endrin	.0198 ± .0026	5.03
Malathion	.0283 ± .0026	3.27
Hercules AC-528	.0627 ± .0020	5.85
Diazinon	.0583 ± .0111	3.55
Chlorthion	.1120 ± .0072	10.60
Toxaphene	.3372 ± .0329	5.58
Pyrazoxon	.3724 ± .0520	4.04

tested. Other materials in the order of toxicity were Bayer 17147, endrin, malathion, Diazinon and Hercules AC-528, Chlorthion, and toxaphene and Pyrazoxon.

Field Experiments.—Results of experiments 1, 2, and 3 are shown in table 2. In experiment 1, 0.2 pound of malathion and 0.1 pound of endrin applied per acre significantly reduced the brown cotton leafworm infestation 3 days after treatment. The other compounds tested in this experiment were not effective. Nine days after treatment there was no difference in the number of larvae on treated and untreated plots. In experiment 2, the use of both levels of parathion and malathion resulted in a significant reduction in the number of brown cotton leafworms. There were no significant differences in the percentage of living larvae among plots treated with the high and low levels of the two compounds 3 days after treatment. Diazinon applied at the indicated dosages did not effectively control the pest.

Results of experiment 3 are shown in table 2. Effective control was obtained with all treatments for 6 days. There was no difference among treatments. As a result of very heavy egg-laying in the experiment, a large number of small larvae developed in all plots by the ninth day; however, the use of all materials except malathion resulted in a significant reduction of the population over the check.

CONCLUSIONS.—Parathion was the most effective compound tested against the brown cotton leafworm, *Acontia dacia*, in the laboratory. Other materials in the order of toxicity were Bayer 17147, endrin, malathion, Diazinon and Hercules AC-528, Chlorthion, and toxaphene and Pyrazoxon.

Applications of parathion, endrin, malathion, Chlorthion, toxaphene, Bayer 17147, and Hercules AC-528 at

Table 2.—The percentage living of *Acontia dacia* Druce following the use of insecticides in field tests at College Station, Texas. 1955.

MATERIALS	POUNDS ACTUAL PER ACRE	AVERAGE PERCENTAGE LIVING ON DAYS AFTER APPLICATION				
		1	2	3	6	9
Experiment 1						
Aldrin	0.125			107.5		52.8
Toxaphene	.75			72.8		23.0
BHC (g.i.)	.15			81.0		53.0
Malathion	.2			1.3		32.8
Heptachlor	.125			154.5		57.0
Endrin	.1			3.5		15.5
Check				92.5		81.8
L.S.D. at 5% level				88.5		n.s.
Experiment 2						
Parathion	0.125			7.8		
	.0625			25.5		
Diazinon	.125			40.3		
	.0625			43.8		
Malathion	.2			10.3		
	.1			12.5		
Check				79.3		
L.S.D. at 5% level				42.4		
Experiment 3						
Toxaphene	2.5	13.5	2.0	6.5	47.5	53.0
Malathion	0.25	3.3	6.3	14.8	50.8	85.8
Endrin	.2	8.5	5.0	8.5	34.5	39.0
Parathion	.0625	3.0	1.3	5.0	34.8	37.5
Chlorthion	.5	4.6	2.5	6.3	55.8	51.5
Bayer 17147	.1	6.8	8.5	6.8	61.3	66.0
Hercules AC-528	.3	17.0	21.5	19.5	53.3	51.5
Check		106.0	115.0	105.0	144.8	110.5
L.S.D. at 5% level		34.65	34.94	30.99	45.34	44.30

the dosages indicated in experiment 3 gave effective control of the brown cotton leafworm in the field up to 6 days after application.

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Control of Some Insects Which Damage Roots of Sweet Potatoes in the Field[1]

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Control measures for insects which damage sweet potato roots in the field in Louisiana were for many years directed primarily against the sweet potato weevil, *Cylas formicarius elegantulus* (Sum.). Gradually, through field experiments begun in 1941 by the Louisiana Agricultural Experiment Station, it was shown to be economically feasible to protect sweet potatoes from injury by soil fauna which mar the quality and grade of the roots (Floyd 1955).

Soil treatments with insecticides were directed against such miscellaneous insects as white grubs, *Phyllophaga* spp., wireworms, *Conoderus* sp., flea beetle larvae, mostly *Chaetocnema confinis* Crotch, banded cucumber beetle larvae, *Diabrotica balteata* Lec., and mole crickets of undetermined species. An application of granulated 5% aldrin at 2 pounds of toxicant per acre was shown to give protection to sweet potato roots from injury caused by these pests. It also gave good control of light infestations (10 to 15%) of the sweetpotato weevil.

The development of new planting equipment and techniques has opened possible new avenues of approach to methods of application of insecticides to the soil. Present equipment can give a uniform distribution of insecticides in the planting row. Also, sweet potato planting machines are now equipped with watering devices. Use of these means should facilitate application of insecticides to the soil. Experiments continued through the 1955 growing season were, therefore, expansions of previous studies of insecticides, methods of application, dosages and formulations to determine those most fitted to the growers' needs. Results of six soil insecticide tests completed in the major sweet potato growing areas of Louisiana in 1955 are reported herein.

PROCEDURE.—All treatments were made to plots three rows wide in a randomized block design of four replications. Row lengths varied with the individual tests, ranging from 100 to 300 feet. Plant spacings within rows were 12 inches. Rows were 4 feet wide. Varieties, planting dates and locations of tests are given in the tables.

One hundred sweet potatoes from the center row of each replicate in each of the tests were examined at harvest for damage by insects. Severity of injury was also recorded in some tests. Severity index ratings, where they are presented in tables, were derived by multiplying the numbers of sweet potatoes in each rating per treatment replicate by their respective rating number. The sums of the ratings for each treatment replicate were divided by the highest severity rating employed. Ratings ranged from 0 to 4 with 0 representing no damage, number 1 representing sweet potatoes with one emergence hole or feeding injury. Number 2 represented those showing from 2 to 5 separate insect injuries; number 3 had 6 to 10; number 4 over 10. Severity indices determined are on a percentage basis.

Five per cent aldrin granules[2] were applied in a 6 to 8 inch band over the center of the partially prepared row with a one-row McCormick fertilizer distributor. The insecticide was then chopped into the soil, and ridging of the rows was completed with hilling disks. Rows were then leveled for planting. The granules were thus somewhat dispersed in the top 4 inches of the soil. The aldrin was applied at rates of 1.0, 1.7 and 2.0 pounds per acre. The same technique was used with 2½% heptachlor granules[3] applied at rates of 1.7 and 2.0 pounds per acre.

Aldrin 25%, wettable powder was pre-mixed with fertilizer and applied at 1.7 and 2.0 pounds per acre. Heptachlor-fertilizer mixtures were prepared from an emulsion concentrate containing 2 pounds of toxicant per gallon. In this mixture heptachlor was applied to the row at rates of 1.7 and 2.0 pounds per acre. Application was made with the fertilizer distributor, the mixture being placed in a 2-inch band 2 to 4 inches deep in the partially prepared row. After the row preparation was complete, the fertilizer-insecticide mixture was 4 to 6 inches below the surface.

Water-spray formulations were applied on the surface of the partially prepared row by a hand sprayer, in 12- to 16-inch bands. These, as were all the treatments, were immediately chopped into the soil as the rows were prepared.

In one test with fertilizer-starter water a dosage of 2 pounds of aldrin, (25% WP) per acre was added to plant

[1] Accepted for publication April 13, 1956.

[2] Shell Chemical Corporation, New York 20, N. Y.

[3] Velsicol Corporation, 330 E. Grand Avenue, Chicago 11, Ill.

Table 1.—Effect of insecticides on per cent of sweet potato roots damaged by soil insects at harvest. Sweet Potato Research Center, Chase and Jessey Miller Farm, St. Landry Parish, 1955.

Treatments	Per Cent of Root Injured	
	Chase Research Center[a]	St. Landry Parish[b]
5% aldrin—granulated	4.0	1.8
2½% heptachlor—granulated	3.5	0.5
25% aldrin, WP, pre-mixed with fertilizer	5.8	2.3
Heptachlor, EC (2 lbs./gal.) pre-mixed with fertilizer	6.5	6.5
25% aldrin, WP[c]	6.5	1.0
Heptachlor, EC (2 lbs/gal.)[c]	2.3	0.5
Check	40.8	6.8
L.S.D. at 5% level	13.25	4.09
at 1% level	18.18	

[a] All insecticides were applied at 1.7 pounds per acre. Plants were Goldrush variety set May 12.
[b] All insecticides were applied at 2 pounds per acre. Plants were Unit 1 Puerto Rico set May 15.
[c] Sprayed on soil in 27 gallons of water per acre.

starter water. Dieldrin[2] emulsion concentrate containing 1½ pounds per gallon was applied in the starter water at a rate of 2 pounds of dieldrin per acre. One-half pint of the insecticide-starter water solution was applied to each plant.

Studies on the residual effectiveness of aldrin and chlordane[3] applied to the soil in a single application in 1953 were completed. Results obtained from this application of 5% materials, in granulated form, made at the rate of 3 pounds of toxicant per acre were recorded for 1953–55.

RESULTS.—The degree of infestation of sweet potato fields by soil insects varied considerably in the test areas. Most of the injury appeared to result from white grubs, larvae of the banded cucumber beetle and flea beetle larvae, their respective importance being in the order named. Wireworms did not appear to be a serious problem on sweet potato roots in the tests made during 1955. Per cent of damage to sweet potato roots in the untreated plots by soil insects varied from 6.8% (table 1) to 98.0% (table 2) in the various locations observed. In tests where

Table 2.—Effect of insecticides on per cent of sweet potato roots damaged and severity of injury by soil insects at harvest. Port Hudson, Louisiana, 1955.[a]

Treatments	Per Cent Injured	Severity Index
5% aldrin, granulated	20.5	11.4
2½% heptachlor, granulated	6.3	3.2
25% aldrin, WP, pre-mixed with fertilizer	76.3	48.2
Heptachlor, EC (2 lbs./gal.) pre-mixed with fertilizer	64.8	41.1
25% aldrin, WP[b]	6.8	3.5
Heptachlor, EC (2 lbs./gal.)[b]	4.3	2.4
Check	98.0	74.6
L.S.D. at 5% level		8.51
at 1% level		11.67

[a] Insecticides were applied at 2 pounds per acre. Plants were Unit 1 Puerto Rico set June 15.
[b] Sprayed on soil in 27 gallons of water per acre.

Table 3.—Effect of starter solutions containing insecticides on injury to sweet potato roots by soil insects and by sweet potato weevil. Baton Rouge, Louisiana, 1955.

Treatments[a] Starter Solutions	Injury by Soil Insects		Per Cent Injured by Sweetpotato Weevil
	Per Cent	Severity Index	
Check—no treatment	45.2	29.2	2.4
Water only	44.2	25.2	6.8
3 lbs. of 10-52-17 fertilizer per 50 gal. water	33.8	18.4	9.8
Soil sprayed with 2 lb. aldrin/A. +water[b]	7.8	3.9	0.8
Soil sprayed with 2 lb. dieldrin /A.+water[b]	11.6	5.7	1.6
2 lb. aldrin/A. in water	18.4	9.1	0.6
2 lb. dieldrin/A. in water	12.0	6.3	1.2
41 lb. 10-52-17 fertilizer+2 lb. aldrin in 50 gal. water/A.	17.2	9.1	0.4
41 lb. 10-52-17 fertilizer+2 lb. dieldrin in 50 gal. water/A.	12.6	6.2	2.0
L.S.D. at 5% level		5.79	4.67
at 1% level		7.76	

[a] All plant starter solutions were applied at ½ pint per plant. Variety was Goldrush set June 22.
[b] Only water used as starter solution.

the number of sweet potato roots damaged resulted in less than approximately 50%, no significant differences could be found among the various insecticides, dosages or methods of application (table 1). Under these conditions aldrin and heptachlor in granulated formulations, fertilizer-insecticide mixtures, and soil surface spray applications all gave good control at the different dosage levels tested. In a dosage test with 5% aldrin granules on Unit 1 Puerto Rico plants set June 15 on the Eric Brasseux Farm, St. Landry Parish, the untreated check had 55.8% of the roots damaged whereas the areas treated with the 1-pound and 2-pound dosages of the toxicant had only 3.8% and 2.5%, respectively, of the roots damaged. Two pounds of aldrin or dieldrin in the plant starter water (table 3) gave good control when damage in the checks was less than 50%. However, when injury in the checks neared 100% (table 2), differences in effectiveness among the methods of application became pronounced (also figure 1). Applications of granules or sprays gave the better results, with the sprays being somewhat superior. Insecticide-fertilizer treatments gave 23.7% control of injury to sweet potato roots (table 2) when 2 pounds of aldrin were applied pre-mixed with fertilizer. In the same test heptachlor pre-mixed with fertilizer at this rate

FIG. 1.—Sweet potato roots showing contrast of insect injuries in treated soils versus check plots. Sweet potato roots on bottom row are from check. Field experiment, Port Hudson, Louisiana 1955.

Table 4.—Residual effect of a single application of insecticides[a] against soil insects and sweetpotato weevil. Baton Rouge, Louisiana, 1953–55.

	Per Cent Injury to Sweet Potato Roots					
	Chlordane		Aldrin		Check	
Year	Soil Insects	Sweet-potato Weevil	Soil Insects	Sweet-potato Weevil	Soil Insects	Sweet-potato Weevil
1953	5.7	9.0	0.5	4.8	13.5	12.2
1954	2.0	41.0	1.0	32.0	33.0	45.0
1955	73.5	21.3	50.3	9.0	98.5	34.5

[a] 5% granulated materials were applied in 1953 at 3 pounds of toxicant per acre.

protected 35.2% of the roots. Under these same conditions 2 pounds of granulated aldrin per acre gave 80% control; 2 pounds of heptachlor in granules, 93.7%; 2 pounds of aldrin in the spray formulation, 93.2% and 2 pounds of heptachlor in spray formulation, 95.7%.

At no time during the growing period did any of the various insecticides tested show any adverse effects on the normal growth of the plant.

Studies of residual effects of insecticides (table 4) for control of insects affecting sweet potato roots showed that 3 pounds of aldrin or chlordane applied in the row did not remain economically effective for more than 2 years. In these tests aldrin gave better results over the 3-year period than chlordane.

In areas infested with sweet potato weevil some data on control of this insect were obtained from the soil insecticide tests which were designed primarily for control of soil insects. Some degree of sweetpotato weevil control was obtained in all of the tests in which 2 pounds of aldrin or dieldrin were added to the fertilizer-water starter solutions (table 3). In this test the weevil indicated a preference for the healthier plants, as shown by infestation among the check treatments, viz., 2.4% root injury where no water was applied to the plants when set as compared with 6.8% for water treatment only and 9.8% for the treatment consisting of 3 pounds of fertilizer in water (table 3).

From these data it appears that the type of formulation and the method of application of soil insecticides are important factors in the successful control of insects which affect sweet potato roots in the field.

Summary.—Field experiments were conducted in 1955 for the purpose of testing the effectiveness of several insecticides in protecting sweet potato roots from damage caused by certain species of soil insects. White grubs, *Phyllophaga* spp., larvae of the banded cucumber beetle, *Diabrotica balteata* Lec., and flea beetle larvae, mostly *Chaetocnema confinis* Crotch, appeared to cause the major portion of damage to the roots. Heptachlor, aldrin, chlordane, and dieldrin applied previous to planting were tested in various methods of application, dosages, and formulations. All the chemicals gave good control, resulting in the production of undamaged sweet potatoes when infestations in the checks were less than 50%. However, when injury in the checks neared 100%, differences in effectiveness among the methods of application became pronounced. Granulated and spray formulations gave the better results, with the spray method somewhat superior. Studies on residual effect of soil treatments with granulated chlordane and aldrin indicated that economic control of soil insects attacking sweet potatoes cannot be obtained for more than 2 years after the initial application.

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Experiments on Control of the Tomato Russet Mite in Southern California[1]

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The tomato russet mite, *Vasates lycopersici* (Massee), has been a serious pest of tomatoes in California since 1940. It gradually spread eastward until by 1955 it was found in 24 states. It has also been reported from Canada and several other countries. The literature of this mite has been reviewed recently (Anderson 1954, Wilcox & Howland 1954). This paper reports the results of control experiments conducted in southern California from 1942 to 1955.

Methods.—The experiments were conducted in commercial fields of tomatoes on small plots, usually 50 feet long and 6 rows wide (rows 5 to 7 feet apart), and were arranged in randomized blocks. All experiments had three to seven replicates except one, which had two. Dust applications were made with rotary hand dusters. In the spray tests semiconcentrated sprays were applied to the small plots with a high-pressure hand sprayer and to the large plots with a power sprayer having a fixed boom covering five rows at a time. *Tee-jet* nozzles giving a fine mist spray were used at 150 pounds' pressure. In most of the experiments the test mixtures were selected to control either the tomato fruitworm or leaf miners or both. The applications were usually scheduled as for the control of the tomato fruitworm—i.e., three applications at 2-week intervals beginning when 5 to 10 tomatoes were set per plant.

Mite counts were made on five or more leaves picked at random from five plants from the central part of each plot. Populations were computed on the basis of the number of mites found on 100 sq. mm. of leaf surface.

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Counts were made several times during the season, if possible. However, it was sometimes necessary to treat with sulfur as soon as possible after the first count, since informal agreements with the growers required that the crop be protected from damage by the russet mite.

TESTS WITH SULFUR.—In 1942 two experiments were conducted with sulfur at several strengths and in several schedules of application. The first experiment was made in a small field of late tomatoes adjacent to an early field infested with mites. In one group of tests a pre-fruitworm application of sulfur alone was followed by one to three applications of a dust containing 25% of sulfur in natural cryolite, and in another the same schedule of fruitworm applications of a cryolite dust containing 50% of sulfur but without the pre-fruitworm application. Owing to cool weather, mite populations accumulated slowly. The mites present on 10 leaves per plot were counted on November 24 and 25 and December 9. There was no severe mite damage. As shown in table 1, two or more applications of the cryolite-sulfur gave excellent control (above 90%) whether or not the pre-fruitworm application was included.

In another experiment in 1942 with a single application of a sulfur dust mite control increased slightly, from 64 to 84%, as the strength of sulfur was increased from 25 to 100%. A commercial dinitro dust (1.5% of dicyclohexylamine dinitro-*o*-cyclohexylphenate and 1.8% of petroleum oil in talc) gave 61% reduction of the mites, but severely burned the plants in one of two fields in which it was tested.

In 1943 tests were made of five brands of sulfur selected to give a range of particle sizes from 4 to 19 microns surface mean diameter. There was no appreciable difference in the effectiveness of the five brands when used at 25% strength in three applications 2 weeks apart. The dinitro compound without the mineral oil, also included in this experiment, gave very poor control when used at 0.5, 1.0, and 1.5% and was inferior to sulfur.

In 1943 sulfur at strengths of 25, 50, 75, and 100% gave 95, 96, 99, and 99% control, respectively, when applied three times 2 weeks apart, each at 30 pounds per acre. Also in this experiment comparisons were made of one, two, and three applications. Additional evidence was obtained that two applications are needed and that three are more likely to give adequate control.

In 1943 growers mounted on a truck an orchard duster powered with a 22-hp. engine, with three fishtail outlets extending to one side at 1, 5, and 10 feet. As the truck was driven up and down the fruit-pickup rows, 20 rows apart, the dust was blown over 10 or more rows very rapidly. Forty acres could be dusted in 3 hours by this method. Observations by county agricultural inspectors during the dusting indicated that the coverage was satisfactory across the entire 20 rows, but later observations indicated good control only on the 5 rows on each side while the center 10 rows were visibly damaged. On September 8 records were made of mite populations in a field that had received at least three applications of 25% sulfur during July and August, each at the rate of 25 pounds per acre. Based upon the four center rows as a check, control ranged from 85 to 99% in the first five rows from the duster, 46 to 70% in the next three rows, and 60% in the drive row.

TESTS WITH OTHER INSECTICIDES.—In 1944 large numbers of russet mites were found in the plots dusted with 2.5, 5, and 10% DDT at 30 pounds per acre on August 31 and September 13 and 27. In the experiments to follow DDT was therefore only given the same consideration as a diluent in interpretion of the value of the dusts or sprays in controlling the mites.

In 1946 chlordane and BHC dusts were tested in comparison with dusts that contained 25% of sulfur plus either DDT or TDE. Applications were made on August 14 and 27 and September 10, each at 30 pounds per acre. Russet mite damage began to appear in the untreated plots by September 25, when the records shown in table 2 were made. All the dusts were effective. The sulfur dusts and 5% chlordane without sulfur were significantly better than 2% chlordane or BHC containing 2.5% of gamma isomer. An application of 25% sulfur was made after the examination, and no further trouble from russet mites developed in this field.

In 1949 tests were made with several insecticides, some of them with 25% of sulfur. The plots were 3 rows wide (rows 5 feet apart) and 66 feet long and separated laterally by young orange trees in a 14-foot strip. The following materials were applied at 30 pounds per acre on August 30 and September 14 and 28 (figures indicate per cent): DDT 10 plus sulfur 25, dieldrin 2.5, calcium arsenate 100, fluorine analog of DDT 10 plus sulfur 25

Table 1.—Control of tomato russet mite with several strengths of sulfur diluted with cryolite and different schedules of application. El Modena, Calif., 1942.

PER CENT OF SULFUR USED ON—				TOTAL POUNDS OF DUST PER ACRE	MITES PER 100 SQ. MM. OF LEAF	PER CENT CONTROL
Sept. 5	Sept. 18	Oct. 3	Oct. 19			
100	—	—	—	15	10.1	40
100	25	—	—	41	4.3	75
100	25	25	—	68	1.3	93
100	25	25	25	96	0.2	99
—	50	—	—	25	7.7	55
—	50	50	—	57	1.0	94
—	50	50	50	96	0.2	99
Untreated check				—	17.0	—
L.S.D. (5% level)				—	2.4	—

Table 2.—Comparison of sulfur, chlordane, and BHC dusts against the tomato russet mite, 1946.

MATERIAL (PER CENT)	MITES PER 100 SQ. MM. OF LEAF	PER CENT CONTROL
Sulfur 25 plus—		
TDE 10	0	100
5	1	99
DDT 10	1	99
Chlordane		
5	3	97
2	34	61
BHC, gamma isomer		
5	17	80
2.5	35	60
Untreated check	87	—
L.S.D. (5% level)	31	—

Prolan 2.5 plus sulfur 25; aldrin 2.5; and Bulan 2.5 plus sulfur 25. On September 14 all plots that were not treated on August 30 with dusts that contained sulfur developed a serious mite infestation, with damage already evident in the center of the plants. These plots were dusted immediately with undiluted sulfur at 30 pounds per acre. Up to the end of the harvest season, on November 15, no further damage was evident in these plots or those treated with 25% sulfur plus an insecticide.

In 1950 excellent control was obtained with suspension sprays containing 3 pounds of toxaphene or chlordane, 1½ pounds of dieldrin, ½ pound of EPN, or 4 pounds of schradan per 40 gallons applied at 26 to 36 gallons per acre on August 28 and September 6 and 19. By October 16 the check plots were severely damaged by an average of 44 mites per 100 sq. mm., whereas no mites could be found in the treated plots. Cane sugar at 10 pounds per 40 gallons was added to the toxaphene, chlordane, and dieldrin sprays to increase their value against dipterous leaf miners. The effect of the sugar on mite control is not known. Later tests on the value of sugar in the sprays failed because of low infestations.

In 1953 tests with chlorinated hydrocarbon and organic phosphorus dusts were continued in two fields. Applications were made in the first field on July 9 and 28 and August 6, and in the second field on July 16 and 30 and August 14. Mite records were made in the first field on October 8, 20, and 22, and in the second field on October 8, 20, and 27 and November 6. As shown in table 3, the dusts that contained toxaphene, DDT plus EPN, or dieldrin were more effective than the sulfur dust, whereas the dusts containing heptachlor or DDT alone were inferior. Isodrin, methyl parathion, and parathion were as effective as sulfur. One of the DDT plots was severely damaged, but no damage was observed in the other plots.

Table 3.—Tests of chlorinated hydrocarbons and organic phosphorus insecticides as dusts against the tomato russet mite, 1953.

Material (Per Cent)	Mites per 100 Sq. Mm. of Leaf	Per Cent Control
Two early fields (9 replications, 3 applications)		
Toxaphene 20	0.17	98
EPN 2+DDT 5	.49	96
Dieldrin 2.5	.69	94
Isodrin 2	1.72	85
Sulfur 25+DDT 5	2.20	81
Parathion 2+DDT 5	2.22	80
Methyl parathion 2.5	2.55	78
Sulfur 25	2.68	76
Heptachlor 5	6.32	44
DDT 5	11.36	0
L.S.D. (5% level)	1.64[a]	—
One late field (6 replicates, 1 application)		
EPN 2+DDT 5	0.2	99
Toxaphene 20	.3	99
Parathion 2+DDT 5	.5	98
Methyl parathion 2.5	.9	96
Isodrin 2	9.4	62
Dieldrin 2.5	10.5	57
DDT 5	19.4	21
Heptachlor 5	25.5	0
Sulfur 25+DDT 5	33.3	0
Untreated check	24.5	0
L.S.D. (5% level)	9.0[a]	0

[a] Only treatments with readings lower than for DDT alone were included in the analysis.

Table 4.—Control of tomato russet mite and tomato fruitworm with toxaphene substituted for DDT in one or more of the three applications normally applied 2 weeks apart for fruitworm control, 1954.

Applications in Which Toxaphene Was Substituted for DDT	Number of Mite-Infested Leaves Out of 140	Per Cent Reduction in Fruitworm Damage[a]
None	34	96
Third	1	96
First	2	94
First, third	0	94
Second	0	87
First, second	0	87
Second, third	2	86
All	0	76
L.S.D. (5% level)	20	11

[a] Based on 14.5% damage in sulfur-dusted plots in adjoining experiment.

Another test was made in 1953 in an older field that had become severely infested with the russet mite owing to poor application of sulfur by airplane earlier in the season. One application was made with hand equipment to six replicates on October 22. Mite records made on October 27 and 28 and November 4 and 12 are shown in table 3. EPN, toxaphene, parathion, and methyl parathion gave rapid and excellent control; isodrin and dieldrin were inferior; and heptachlor was not effective. The failure of the sulfur dusts was probably due to the unusually low mean temperature of 53.2° F. during this period.

In 1954 it was demonstrated that a single application of toxaphene emulsion would control a light infestation of the tomato russet mite and could be substituted for DDT in any one of the three regular applications for fruitworm control. However, where toxaphene was used for all three applications, less effective control of tomato fruitworm was obtained (table 4). Applications were made with a power mist sprayer at approximately 7 gallons per acre. DDT was applied at 1 pound per acre and toxaphene at 4 pounds. Twenty per cent (by volume) of liquid lime sulfur included in DDT emulsion in one set of plots (seven replicates, not mentioned in table 4), did not adequately control the russet mite.

In a small field of early tomatoes there was evidence that sulfur cannot be relied on for control of russet mites in cool weather. These tomatoes were transplanted in March and were dusted at least three times with 50% sulfur plus 5% DDT prior to July 1, but were damaged by russet mites in July.

In 1955 a large commercial planting transplanted in April and dusted once or twice with 50% sulfur plus 10% DDT prior to July 1 was damaged, and live mites were found in late July. The grower's prompt application of a 20% toxaphene dust prevented further damage. This planting was in a cool area about 2 miles from the ocean.

These observations indicate that the tomato russet mite can build up to damaging numbers at temperatures below those necessary to make sulfur effective. On early plantings in cool localities it would seem advisable to use toxaphene or combinations of DDT with parathion, EPN,

or methyl parathion, all of which were found to be effective under cool weather conditions in 1953 (table 3).

Another incident that occurred in 1955 seems worth recording. Experimental plots in the central part of a 10-acre field of early tomatoes were treated on July 6 and 20 and August 5, with various insecticides other than sulfur. The remainder of the field was treated by the grower at about the same times with 50% sulfur plus 10% DDT. Live mites were found in the experimental plots during August, but after an extremely hot spell early in September, when daytime temperatures averaged 100° F. or more for 10 days, no live mites could be found. It seems probable that these mites were killed by the sulfur fumes blown by the wind into these plots from the surrounding areas.

SUMMARY.—The tomato russet mite, *Vasates lycopersici* (Massee), was first found in California in 1940, and by 1955 had spread to 24 states. Experiments on its control in southern California beginning in 1942 and continuing intermittently through 1955 are reported.

Excellent control was obtained by including 25% of sulfur in dusts applied for control of the tomato fruitworm. There was very little difference in the effectiveness of dusts containing 25, 50, 75 or 100% of sulfur in three applications at 2-week intervals beginning when 5 to 10 tomatoes had set per plant. Inferior results were obtained with only one or two applications.

Toxaphene, chlordane, dieldrin, and EPN were very effective. Parathion, schradan, methyl parathion, and isodrin were also effective in limited tests. Inferior results were obtained with DDT, aldrin, heptachlor, BHC, calcium arsenate, and a dinitro compound.

On plantings grown during cool weather, as in the late fall or early spring, sulfur was not always effective. Toxaphene, EPN, parathion, and methyl parathion were found effective under these conditions, and can be used alone or combined with DDT for tomato fruitworm control.

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Low-Elevation Movement of Some Species of Aphids[1,2]

W. A. SHANDS,[3] G. W. SIMPSON,[4] and J. E. DUDLEY, JR.[5]

Movement of winged forms is a common means by which many crops become infested with aphids, both initially and after treatment with insecticides. Aphids also serve as vectors of several virus diseases of crops. Broadbent & Gregory (1948), Simpson & Shands (1949), Broadbent *et al.* (1950), and Broadbent & Tinsley (1951) have shown that winged aphids are more important than wingless aphids as vectors of some virus diseases of potato. However, Klostermeyer (1953) said it would be hasty to conclude that wingless aphids are of minor importance.

The distance that winged aphids can move doubtless is influenced by the height at which they travel. Working with 12-inch tall, stovepipe-like, sticky screens at three elevations in a field of potatoes, Broadbent (1948) found that 67% of the green peach aphids (*Myzus persicae* (Sulz.)) taken were caught at a mean height of 68 inches, 25 to 28% at 38 inches, and 5 to 8% at 7 inches. Nowak (1951), using sticky drums, captured alate forms of this aphid at heights from 2.5 to 45 meters. From operating windvane traps in strategic places, Gorham (1946) concluded that some species of potato-infesting aphids move at high elevations over wide barriers of forest and water. Johnson (1954), summarizing his studies on aphid flight using suction traps, pointed out that density changes during the day in airborne aphids recorded at altitudes of 5 to 10 feet are often reflected at distances up to 150 feet. During the summer of 1949, Johnson (1953) found that between 8:30 A.M. and 4:30 P.M. 77% of the total airborne population was above 10 feet. Johnson & Penman (1951) found a linear relationship between the logarithm of aphid density and the logarithm of height within the range of 10 to 2000 feet. From airplane-trapping studies in Louisiana, Texas, and northern Mexico, Glick (1939) reported taking 14 specimens of the pea aphid (*Macrosiphum* (=*Illinoia*) *pisi* Harris) at 200 feet, and 1 each at 1,000 2,000 and 4,000 feet; 3 specimens of *Macrosiphum* (=*Illinoia*) spp. at 200 feet; and 1 green peach aphid at 3,000 feet.

Aphid trapping was done in connection with biology studies of potato-infesting aphids in northeastern Maine and of the pea aphid in Wisconsin and New York. A major objective was to obtain data to indicate the importance of low-elevation movement of winged aphids as a means by which fields of potatoes and English peas become infested by these aphids. An additional aim of the work in Maine was to gather data to help assess the role of the winged aphids in spreading certain potato virus diseases from field to field. The trapping in Maine was done in 1942–44, in Wisconsin in 1923–28, and in New York in 1927. Windvane traps were operated in Maine and tanglefoot screens in Wisconsin and New York. This paper reports the height distribution of these aphids as trapped at each place.

EXPERIMENT IN MAINE.—The trapping in Maine was done at one place on Aroostook Farm near Presque Isle by operating a series of windvane traps one above an-

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[2] L. H. Taylor, of the Maine Agricultural Experiment Station, gave valuable advice on the statistical analyses, and Rodney Cecil, formerly of the Bureau of Entomology and Plant Quarantine, tended the adhesive screens at Geneva, N. Y.

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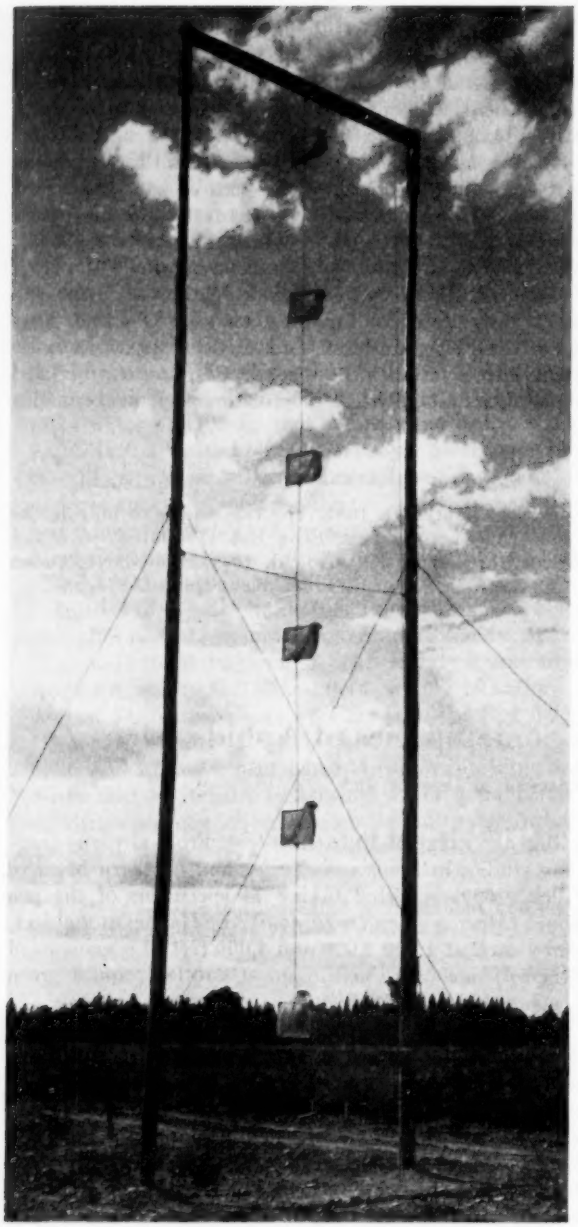

FIG. 1.—The windvane trap assembly operated at Presque Isle, Me.

other on a rope. The trap used was essentially a miniature of the one described by Shands *et al.* (1942). This trap, framed with steel wire, was covered with white cotton scrim and it had an orifice 12 inches square. Two ½-inch loops of steel wire attached to the center front of the top and bottom of the trap opening served as guides for the rope strung through them. Fixed bearing surfaces along the rope held the traps 5 feet apart. In operation, the line of traps (Fig. 1) was suspended vertically between two poles about 8 feet apart connected by two crosspieces, one at the top and one about 4 feet above the ground. The rope, fastened to the middle of the lower crosspiece, ran through a pulley attached to the middle of the upper crosspiece, thence through a pulley at the top of one of the poles, and straight down to the end of the lower crosspiece, where it was fastened. To examine the traps for aphids, the rope was loosened at the end of the lower crosspiece and the traps were lowered one at a time to a height convenient for removing the aphids with an aspirator. The collection from each trap was preserved separately, and identification and counting of the specimens were done with the aid of a binocular microscope.

The trapping location was exposed to a clear sweep of wind from the north, south, and west, but two small buildings and a small orchard lay about 60 yards to the east. Except for a 1-acre field of weeds to the southeast, the trapping location each year either was surrounded by potato fields or potatoes were growing immediately to the north and west. During each of the 3 years the experiment was conducted the traps were emptied daily for over a month in August and September, the period when flying aphids were most abundant.

The numbers of winged potato-infesting aphids taken during three seasons at Presque Isle are given in table 1, and the percentages of total catch of each species at different heights in figure 3. Although the buckthorn aphid (*Aphis abbreviata* Patch) was most abundant for the entire period, the green peach aphid predominated in 1944. The potato aphid (*Macrosiphum solanifolii* (Ashm.)) was the least abundant both on potatoes and in the traps.

Analysis of variance showed some significant differences between heights in the numbers of winged aphids caught. However, for critical comparisons of this nature, Williams (1937) showed that more consistent results were obtained by using geometric means than arithmetic means. The arithmetic mean of the $\log_{10}(n+1)$ is the basis used in the following consideration. Analysis of variance was used to detect differences in height distribution, testing *height* against *days*×*height*. Differences

Table 1.—Numbers of winged aphids taken in traps operated one above the other at Presque Isle, Maine, 1942–1944.

HEIGHT OF TRAP (FEET)	APHID		
	Buckthorn	Green Peach	Potato
	Aug. 16 to Sept. 26, 1942		
6	2591	1837	119
11	3432	2198	119
16	3776	3136	111
21	3788	2874	115
26	2653	2058	94
	Aug. 13 to Sept. 18, 1943		
5	828	70	130
10	2234	121	242
15	2390	140	320
20	2207	119	323
25	1579	107	301
30	1009	86	228
	Aug. 6 to Sept. 14, 1944		
5	8769	6615	437
10	9664	9233	877
15	5884	8734	907
20	7831	9678	1113
25	4982	8386	1051
30	3637	6812	828

cited, unless otherwise indicated, are significant for a probability of 0.05.

Table 2 contains the results of the more critical comparison of geometric means for seasonal aphid catch at the several elevations. For the buckthorn aphid it shows that in 1942 the most winged forms were caught at 21 and 16 feet. Fewer were taken at 26 feet than at 11 feet, and still fewer at 6 than at 26 feet. In 1943 the largest catches were at 10, 15, and 20 feet (not differing), and fewer at 25 than at 20 feet. While the catch at 5 feet was smaller than at 25 feet, it did not differ from that at 30 feet. In 1944 largest numbers were caught at 10, 15, and 20 feet, with fewer at 20 feet than at 10 feet. While the catch at 30 feet was smaller than at 5 or 25 feet, the catches at these heights were not smaller than at 20 feet. The catch at 5 feet did not differ from that at 25 feet. The combined data for 1943 and 1944 show that fewer were caught at 25 or 5 feet than at 10, 15, or 20 feet, and the catches were smaller at 30 than at 25 feet. The catch at 10 feet was larger than that at 20 feet.

In 1942 the relative height distribution for the green peach aphid was essentially the same as that for the buckthorn aphid. In 1943, under conditions of very low populations, the numbers taken at the several elevations did not differ significantly. In 1944, not differing among themselves, the largest catches were at 20, 25, 15, and 10 feet. The catch at 30 feet was less than at 10 feet and that at 5 feet was smaller than at any other height. The combined data for 1943 and 1944 were similar to those for 1944.

In 1942 no significant differences between numbers of the potato aphid caught at different heights were found. In 1943, although not differing among themselves, largest catches were at 20, 25, and 15 feet. At 15 feet, however, the catch was not significantly larger than at 30 or 10 feet. The catch at 5 feet was smaller than at any other height. In 1944 the results were rather similar to those of 1943 except that the catch at 15 feet was slightly larger than at 25 feet. The combined data for 1943 and 1944 were similar to those for 1943, except that the catch was significantly larger at 15 than at 30 feet.

Further study of the combined data for 1943 and 1944, when the trapping heights were the same, disclosed additional facts of interest. The general pattern of height distribution differed between days during the 2 years for the green peach and potato aphids, but not for the buckthorn aphid. It also differed between the 2 years for the green peach and buckthorn aphids, but not for the potato aphid. Considering all days of these 2 years, each species exhibited its own rather definite pattern of height distribution.

Table 2.—Mean $\log_{n+1}$ of the number of winged aphids per trap taken daily at several heights in one location near Presque Isle, Maine, 1942–44.

Height (Feet)	Aphid		
	Buck-thorn	Green Peach	Potato
	1942		
6	2.4039	2.1453	0.2835
11	2.7785	2.5728	.3003
16	2.9884	2.7530	.2929
21	3.0494	2.8738	.2958
26	2.6637	2.4336	.2512
L.S.D. for P=0.05	0.0933	0.1358	NS
P=0.01	.1231	.1792	—
	1943		
5	0.9051	0.3531	0.4466
10	1.2186	.4151	.6194
15	1.1760	.4583	.7071
20	1.1694	.4223	.7440
25	1.0526	.3677	.7174
30	.9494	.3669	.6049
L.S.D. for P=0.05	.1083	NS	.1093
P=0.01	.1430	—	.1443
	1944		
5	1.7837	1.9320	0.8874
10	1.9100	2.1271	1.1411
15	1.8200	2.1326	1.1791
20	1.8031	2.2086	1.2314
25	1.7263	2.1654	1.1711
30	1.5997	2.0429	1.1186
L.S.D. for P=0.05	.1053	.0820	.0917
P=0.01	.1391	.1083	.1211
	1943 and 1944 (combined)		
5	1.3445	1.1426	0.6670
10	1.5643	1.2712	.8803
15	1.4980	1.2955	.9432
20	1.4863	1.3155	.9877
25	1.3895	1.2666	.9443
30	1.2746	1.2049	.8617
L.S.D. for P=0.05	.0774	.0801	.0802
P=0.01	.1022	.1059	.1059

EXPERIMENT IN WISCONSIN.—Trapping was done at two places in Wisconsin, near Columbus and near Waterloo. The sticky screen used there (Fig. 2) was similar to that described by Rockwood (1926). Essentially it consisted of 12-mesh black screen wire 30 inches wide that was covered with tanglefoot and held in a vertical position. The mechanism for holding the screen in place differed somewhat depending upon its height. The upper end of the screen was fastened to a band-iron frame suspended by a rope through a pulley attached to a rigid arm extending from the top of a pole or to the top crosspiece between two poles. A second pulley at the top of the pole served to guide the rope downward for fastening to a cleat near the bottom of the pole. The lower end of the screen was attached to a revolving drum equipped with a crank. By simultaneously maneuvering the rope and crank the screen could be lowered for identifying and counting the adhering pea aphids. Both sides of the screens were painted with tanglefoot thinned with alcohol. To insure sticky surfaces, a fresh coat was added as needed, usually every week or 10 days. Once or twice each year the accumulation of tanglefoot and adhering matter was burned from the screens and fresh adhesive applied.

All screens faced east-west except in 1928, when the one near Columbus faced north-south. The aphids stuck to them were identified and counted at intervals of 2 to 4 days. Records were made to show the number of pea aphids on each vertical foot of screen, with the 12-inch distance centered at the designated height. The adhering aphids were then removed or crushed and covered by the adhesive. The screens differed in height from one year to another, but the lower end was always $2\frac{1}{2}$ feet above the soil level. In some years the screens extended to a height

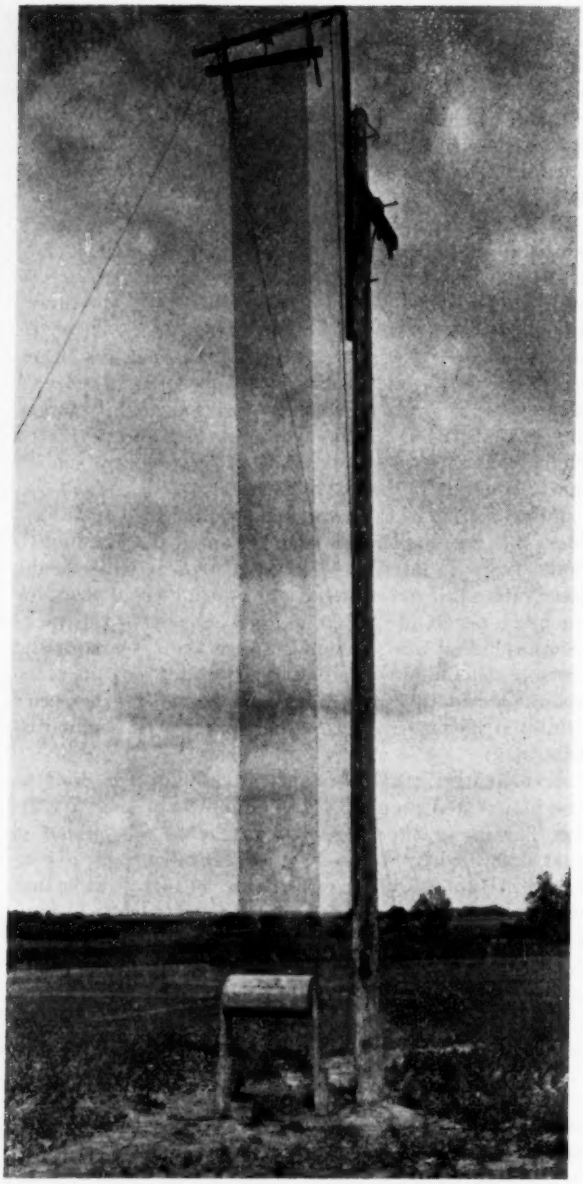

FIG. 2.—One of the adhesive screens operated near Madison, Wis.

of 34½ feet, but in this study catches above the 29½-foot level were excluded since that was the height of the screens some of the years. Duration of the trapping period varied markedly between locations and from year to year. In general, however, it extended each year during the peak pea aphid flight. Specific location of the screens varied from year to year, but usually they were situated on low knolls bordering fields of English peas with no nearby obstructions to wind movement. The bottom of the screens was slightly above the tops of the pea plants.

Table 3 and figure 3 summarize the results from Wisconsin. There were large differences from year to year in total pea aphid catch both within and between trapping locations. These differences were doubtless due in part to differences in overall abundance and in part to unequal

Table 3.—Catches of winged pea aphids on 29-foot tanglefoot screens in two locations near Madison, Wis., 1923–28.

Height (Feet)	Number of Winged Pea Aphids		Mean of $\log_{n+1}$ of Yearly Total Catches
	Location 1	Location 2	
3	450	255	1.3825
4	1002	297	1.6350
5	1107	322	1.7540
6	1126	316	1.7350
7	1257	448	1.7983
8	1330	431	1.8017
9	1341	502	1.8267
10	1401	461	1.7933
11	1463	391	1.7933
12	1533	442	1.8308
13	1350	521	1.7767
14	1405	473	1.7975
15	1443	492	1.8342
16	1459	424	1.8042
17	1482	486	1.8708
18	1316	491	1.8192
19	1373	479	1.8108
20	1414	441	1.8150
21	1407	459	1.8083
22	1368	417	1.8008
23	1243	476	1.7933
24	1187	413	1.7733
25	1166	359	1.7158
26	1162	338	1.7267
27	1138	304	1.6417
28	1015	254	1.5658
29	661	212	1.5325

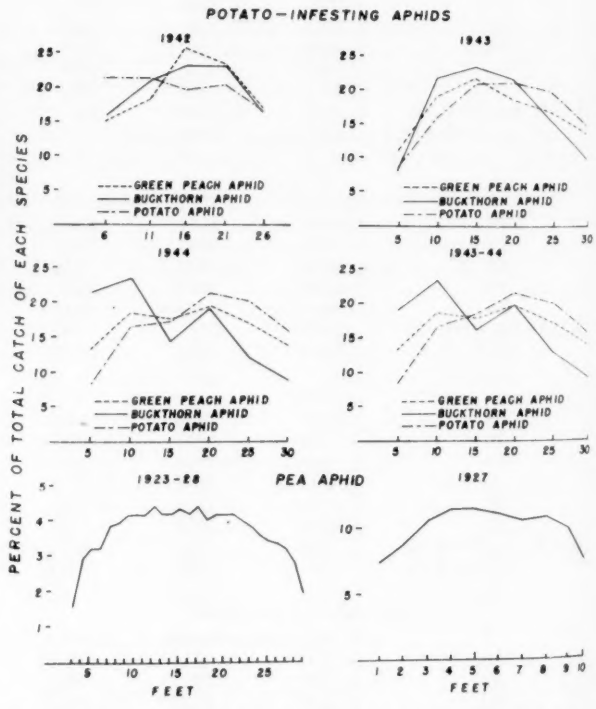

FIG. 3.—Percentages of winged aphids caught at various heights. Determinations for potato-infesting species were made at Presque Isle, Me., and those for the pea aphid in Wisconsin and New York.

periods of screen operation. The total period of operation was 338 days at one location and 233 at the other; and the yearly period ranged from 17 to 103 days at one location, and 7 to 64 days at the other. No tests of significance for differences in height distribution appeared to be justified for these reasons. However, on the basis of the logarithmic transformations the combined mean yearly catches tended to peak at 17 feet, with little difference from 5 to 26 feet.

EXPERIMENT IN NEW YORK.—The procedure for the trapping work in New York differed essentially in only four respects from that in Wisconsin: (1) the tanglefoot screens were operated for 1 year only, 1927; (2) the pea aphids caught on the screens were identified and counted daily from mid-May until August 22; (3) a 10-foot screen was used at all locations with the bottom only $\frac{1}{2}$ foot above the soil level; and (4) one screen was operated at each of three locations (farms) near Geneva.

At locations 1 and 3 (table 4) largest catches of the pea aphid occurred at heights of 3 to 9 feet, and the smallest at 1, 2, and 10 feet, while at location 2 largest catches were from 1 to 6 feet and the smallest from 7 to 10 feet. The results from combining the data for all locations show that largest catches were at 3, 4, 5, 6 and 8 feet (not differing among themselves), with fewer at 7 and 9 feet, and still fewer at 1 and 2 feet. There were fewer at 10 feet than at any other height except at 1 foot. It will be noted that the order of mean logs does not necessarily follow the order for total catches, because of location differences in time distribution of the catches. The height distribution of pea aphid catches at Geneva is shown graphically in figure 3.

The catches varied significantly among days and among trapping locations. The combined influence of days and locations was highly significant. The height of pea aphid movement also differed tremendously with the day and the trapping location. Apparently the numbers caught, as well as the heights at which they moved, were influenced by the location of the traps. This is not surprising, since our observations, as well as those of other workers, have shown that the number of aphids trapped may be influenced by such factors as the size and general topography of the adjacent field of host crop, the direction of the field from the trap with respect to the prevailing wind, the size and seasonal trend of aphid populations on the nearby host crop, and the nearness of obstructions to wind movement. We have no specific knowledge of these factors as they may have influenced the trapping at Geneva.

Table 4.—Catches of winged pea aphids on 10-foot tanglefoot screens in three locations near Geneva, N. Y., May 21–August 22, 1927.

HEIGHT (FEET)	LOCATION 1	LOCATION 2	LOCATION 3	ALL LOCATIONS
	Total Number			
1	270	147	301	718
2	350	171	325	846
3	382	196	448	1026
4	323	193	588	1104
5	340	171	606	1117
6	323	218	539	1080
7	314	146	570	1030
8	331	164	555	1050
9	328	134	505	967
10	254	109	376	739
	Mean of $\log_{n+1}$ of Daily Catches			
1	0.4246	0.3690	0.5017	0.4318
2	.4656	.4107	.4775	.4512
3	.4822	.4239	.5878	.4980
4	.5171	.4110	.6119	.5133
5	.5234	.3925	.6200	.5120
6	.5339	.4261	.5736	.5112
7	.5188	.3354	.5886	.4810
8	.5375	.3495	.6181	.5017
9	.5227	.3193	.5756	.4725
10	.4708	.2842	.4956	.4169
L.S.D. for P=0.05	.0637	.0552	.0613	.0214
P=0.01	.0838	.0727	.0807	.0282

DISCUSSION OF RESULTS.—Two different concepts of low-elevation aphid movement could be obtained by comparing the results in figure 3 with those from work by Davis *et al.* (1954). Using yellow pan traps (Moericke 1951), placed at heights ranging from ground level to 20 feet, they found that 66% of the winged aphids caught during a 2-year period were taken at ground level, and 15, 9, 6, and 4% in pans at 5, 10, 15, and 20 feet, respectively. Although we had no trap operating at ground level, the average percentages of total catch at 5–6, 10–11, 15–16, and 20–21 feet for the 3 years were 21, 27, 25, and 27, respectively. These differences are due chiefly to differences in the kinds of traps used. Work by Moericke (1950, 1952) and Müller (1953 *a*, *b*) indicates that the yellow pan trap may incite an alighting response and attract the landing aphids, whereas our windvane trap merely strains the aphids from the column of air passing through. The number of airborne aphids passing at a given elevation depends upon their density and the wind speed. The pan trap could be expected to show the greatest concentration of aphids at ground level, since wind speed increases with the elevation and an aphid is unable to land at wind speeds exceeding its speed of flight. Our particular interest was to determine the numbers moving past rather than those conditioned to or capable of alighting at different elevations. The windvane trap permitted this, since it operated on the principle of wind impaction.

The indication is that the height-distribution picture of airborne pea aphid movement gained from operating sticky screens depends upon the height of the screen used. On tall screens the largest numbers were caught at 5 to 26 feet, and on the 10-foot screens between 3 and 8 feet, with sharp reductions both above and below these points. Usually the rate of catch reduction was larger near the top of the screen than near the bottom. That is, the drop in size of catch generally was sharper near the top of the screen than near the bottom, irrespective of screen height. The drop at the bottom probably was due in part to lesser movement of the aphids because of wind eddies, wind shelter, and impediments to flight from vegetation nearby. The sharper drop near the top of the screen was probably due to wind currents and eddies. Tanglefoot screens thus appear to have provided a less satisfactory measurement of the low-elevation movement of the pea aphid than the windvane traps did for similar movement of the buckthorn, green peach, and potato aphids. Apparently the general picture of movement of the pea aphid more

nearly resembles that of the potato aphid than of the green peach or the buckthorn aphid.

The results of these trapping studies suggest that little protection from infestation by winged aphids can be expected in uninfested fields of potatoes or peas that are only short distances from infested fields, particularly when the uninfested field is on the leeward side. Since the winged forms of some of these aphids are important vectors of certain virus diseases, neither can similar separation from infected fields be expected to provide appreciable protection from disease spread in the disease-free fields.

SUMMARY.—The movement of winged aphids at low elevations was studied for several years. For studies of potato-infesting aphids windvane traps one above another were operated near Presque Isle, Maine, in 1942–44, and for the pea aphid tall tanglefoot screens were used at Columbus and Waterloo, Wis., in 1923–28, and shorter ones at Geneva, N.Y., in 1927. Within the vertical 30-foot trapping range, catches of the buckthorn, green peach, and potato aphids peaked at 10–20, 10–25, and 15–25 feet, respectively. Pea aphid catches tended to peak at 17 feet within the 29½-foot elevation sampled in Wisconsin and between 3 and 8 feet within the 10½-foot elevation covered by the New York screens.

Sharply reduced catches near the tops of the screens were probably due to wind currents, and more gradual reductions near the bottoms to wind eddies around the screen and to impediments to aphid movement by nearby vegetation. The results suggest that short separations between fields will give little protection from infestation or virus-disease spread by winged aphids.

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The Occurrence of Cholinesterase in Eggs of the Peach Tree Borer and Large Milkweed Bug and Its Relationship to the Ovicidal Action of Parathion[1]

E. H. Smith and A. C. Wagenknecht,[2] *New York State Agr. Expt. Station, Geneva*

The present paper is the third in a series of studies on ovicidal action of parathion. The first report set forth the role of ovicidal action in field control of the peach tree borer, *Sanninoidea exitiosa* (Say), and established minimum effective exposures (Smith 1954). The observation that treated eggs continued embryonic development almost to the point of hatching led to the second study. Here the effect of treatment on the respiratory rate of developing eggs was determined and in addition susceptibility to ovicidal action at various stages of development was established (Smith 1955). To reconcile the various findings of these studies the following scheme of ovicidal action was tentatively proposed: Parathion gains entry as vapor, younger eggs being more permeable. The toxicant taken up at any stage remains in the egg as such or is metabolized to a more active anti-esterase that persists in the egg. The presumed inhibition of cholinesterase is of no physiological importance until embryonic development nears completion at which time it proves lethal. In the present paper this hypothesis has been further explored by investigations on the occurrence and nature of cholinesterase in developing eggs and the inhibitory action of parathion on these enzymes *in vivo* and *in vitro*. Eggs of two species were employed, the peach tree borer characterized by high susceptibility to ovicidal action of parathion and the large milkweed bug, *Oncopeltus fasciatus* (Dall.), which is not susceptible to ovicidal action at practical concentrations.

Although an extensive literature exists on the physiological role of cholinesterase (ChE) in animals (Augustinsson 1948) comparable studies on insects have only recently been undertaken following the disclosure that inhibition of this enzyme is involved in the toxic action of organophosphorus insecticides. Evidence on the occurrence of the enzyme in insect eggs and its role in ovicidal action is extremely limited (Tahmisian 1943; Chino & Yushima 1953, 1954; Yushima & Chino 1953a, 1953b; Lord & Potter 1951, 1954; Casida 1955). In fact, speculation on ovicidal action of organophosphates is based largely on evidence derived from studies on hatched forms. Actually, ovicidal studies offer a unique approach to the matter of mode of action because the organism undergoes drastic developmental changes in which the enzymes in question are presumably not always present and if present may not yet enter into vital physiological functions.

Materials and Methods.—Eggs of the peach tree borer were obtained by the method previously described (Smith 1955). Milkweed bug eggs were from a laboratory culture maintained at 80° F. and 60 to 80% relative humidity. Abundant supplies of milkweed bug eggs could be readily obtained whereas the seasonal limitation on availability of peach tree borer eggs posed a problem considering the quantities required. The age of the milkweed bug eggs used for tests was controlled by exposing masses of cotton to the females for 24-hour periods. Eggs deposited in the cotton during this period were teased free with a camel's-hair brush and swept into Syracuse watch glasses. Counting was done with the aid of a binocular microscope. Eggs were incubated at 80° F. until used for determinations of enzyme activity. The incubation period under these conditions was approximately 8.5 days for the peach tree borer and 6.1 days for the milkweed bug.

Individual samples for enzyme assay consisted of 2000 peach tree borer eggs and 1000 milkweed bug eggs having an average weight of 110.6 mg. and 212.8 mg., respectively. Breis of eggs were prepared by homogenization in 1.25 ml. buffer (0.15M NaCl, 0.04 M $MgCl_2$, 0.025M $NaHCO_3$) in a Potter-Elvehjem homogenizer and 0.5-ml. aliquots used for individual runs.

Cholinesterase (ChE) activity was determined manometrically using the method of Ammon (1933) by measuring the rate of CO_2 production from bicarbonate buffer in the Warburg respirometer at pH 7.4 and 37° C. The Warburg vessels contained the following reactants in a total volume of 2.0 ml.: in the main compartment, 1.0 ml. of 0.0263M $NaHCO_3$ buffer, 0.5 ml. of insect egg homogenate. The substrates were placed in the side arm. Vessels were held in an ice bath until all of the reactants had been added in order to minimize enzyme deterioration during preparation. Blanks containing enzyme (egg brei) without substrate, and substrate without enzyme were included with each determination as controls for endogenous CO_2 production and non-specific hydrolysis of substrate. Vessels were gassed (5% CO_2—95% N_2) in the bath for 10 minutes while being shaken after which the substrates were tipped in. Readings were made at regular intervals for 3 hours or more.

Substrates used included acetylcholine chloride (ACh), acetyl-β methylcholine (AMeCh) and benzoylcholine chloride (BzCh).

Parathion used in inhibition studies was a technical grade (98.5%) formulated in Cellosolve to give a 42% emulsifiable concentrate. (Orthophos 4, California Spray Chemical Co.)

In vivo treatment of borer eggs was accomplished by exposing them on sheets of filter paper impregnated with parathion emulsion (0.035%) using minimum lethal exposures previously determined (Smith 1955). This method was employed for several reasons: first, it more nearly approximates the type of exposure chiefly responsible for control under field conditions than does direct treatment. Secondly, it avoided the introduction of surface residue to the breis as would have occurred from treatment directed to the chorion. Third, eggs could be manipulated so as to vary the time and duration of expo-

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sure. Milkweed bug eggs were not affected by parathion at practical concentrations whether applied to the chorion or by indirect exposure to residue. In comparative tests using treated milkweed bug eggs, exposure was made to filter paper impregnated with 0.7% parathion.

In determinations involving *in vitro* inhibition parathion was diluted with distilled water and added to the side arm of the Warburg vessels.

ChE activity was expressed as b_{180}, the amount of CO_2 released after 180 minutes, corrected for non-specific hydrolysis of substrate and endogenous CO_2 production. Number rather than weight of eggs was held constant in various runs but the variations in weight of individual samples were not sufficient to introduce appreciable error. Furthermore, where practical, eggs were pooled and aliquots of brei used for comparative tests. The three-hour reaction time was chosen because of the relatively low ChE activity of insect eggs as compared with other sources. The rate of CO_2 release at this time was essentially constant, following an initial lag phase. A deviation from the constant rate occurred with reaction times exceeding four hours suggesting the possibility of bacterial contamination.

RESULTS.—*Relative ChE activity.*—The relative activity of ChE from the two sources using eggs in advanced stages of development is shown in table 1.

These values are low by comparison with sources such as fly and bee heads and cockroach ventral nerve cord (Casida 1955). The contrast is not so great when comparison is made on entire bodies of insects. Eggs of both species are less active than fly bodies but borer eggs show more activity than several species of scale insects (Metcalf *et al.* 1955).

Substrate specificity.—In the current study interest in substrate specificity was two-fold: first and primarily, to select substrates suitable for ChE assay in developing eggs and secondly, as an index to the kind of esterases present. The first point, that of occurrence, is pertinent in establishing the relationship between ChE activity and susceptibility of various stages to ovicidal action. The second point, that of characterization of cholinesterases, is of interest because of its possible relationship to differences in susceptibility of the two species. Recent studies have shown that the properties of acetyl esterases from different insect species vary widely and that these differences in turn relate to specific organo-phosphorus sensitivity. Such differences in cholinesterases can be distinguished broadly by their activity on various substrates and intensive research has recently been devoted to the elucidation of this complex relationship (Babers & Pratt 1950, Casida 1955, Metcalf *et al.* 1955).

Table 1.—Comparative activity of ChE from eggs of the peach tree borer and the large milkweed bug, 0.01 M ACh.

SPECIES	AVERAGE WEIGHT PER SAMPLE (MG.)	MICROLITERS CO_2 PER SAMPLE 3 HRS.	MICROMOLES ACh HYDROLYZED	
			Per Sample per Hour	Per Milligram per Hour
Peach tree borer	44.2	325[a]	4.8	0.108
Milkweed bug	85.1	208[b]	3.0	0.035

[a] Average of 7 determinations.
[b] Average of 5 determinations.

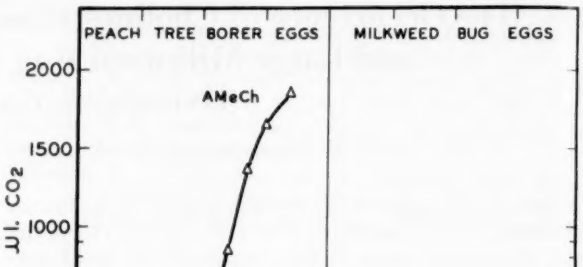

FIG. 1.—Activity—pS curves for enzymatic hydrolysis by peach tree borer and large milkweed bug egg ChE.

Eggs in all stages of development were to be assayed for ChE activity but to conserve test material it was desirable to select a single substrate suited for this purpose. In screening substrates, breis were prepared from eggs in advanced stages of development which presumably possessed near maximum activity. The resulting curves after correcting for non-specific hydrolysis and endogenous CO_2 production are shown in fig. 1.

Both ACh and AMeCh were hydrolyzed at rates sufficiently high to render them satisfactory for assay studies. The results of such studies will be reported later. Regarding substrate specificity, ChE from both sources reacted with ACh to give bell-shaped curves. BzCh was hydrolyzed by enzymes from both sources at a very low rate. Although a concentration series of this substrate was not run with ChE from milkweed bug eggs, a single intermediate concentration showed less activity than with borer egg ChE. Sharp contrast occurred with AMeCh which was readily hydrolyzed by borer egg ChE with no inhibition at high concentrations while the curve for milkweed bug ChE showed less activity and suggested inhibition at higher concentrations.

The contrast in activity curves for AMeCh indicates that cholinesterases from the two sources differ in their properties but the nature of the difference is not apparent from these data. The curves for borer egg brei closely resemble those reported by Metcalf *et al* (1955) for bee heads while the pattern for milkweed bug eggs is more like that of the house fly. These workers concluded that bee- and fly-head breis represent two distinct types of enzymatic activity toward ACh and AMeCh.

The activity curves for milkweed bug eggs are similar to those for milkweed bug heads (Metcalf *et al.* 1955) suggesting that in this species the properties of ChE remain essentially the same during different life history stages. This differs from the findings of Casida (1955) where a qualitative change occurred in different stages of the house fly.

Occurrence of cholinesterase in developing eggs.—While

ACh and AMeCh were both hydrolyzed sufficiently by ChE from both sources to prove satisfactory for assay studies the possibility exists that esterases hydrolyzing BzCh might occur only in early stages. In this case they would have escaped detection in the screening procedure using eggs in advanced stages. Accordingly, the three substrates were included in assays on milkweed bug eggs, the enzyme source more readily available. The substrate used with peach tree borer eggs was limited to ACh which gave similar curves with ChE from both sources. A substrate concentration of 0.01M was adopted based on the activity curves shown in fig. 1. The activity curves for eggs of various ages are shown in figs. 2 and 3.

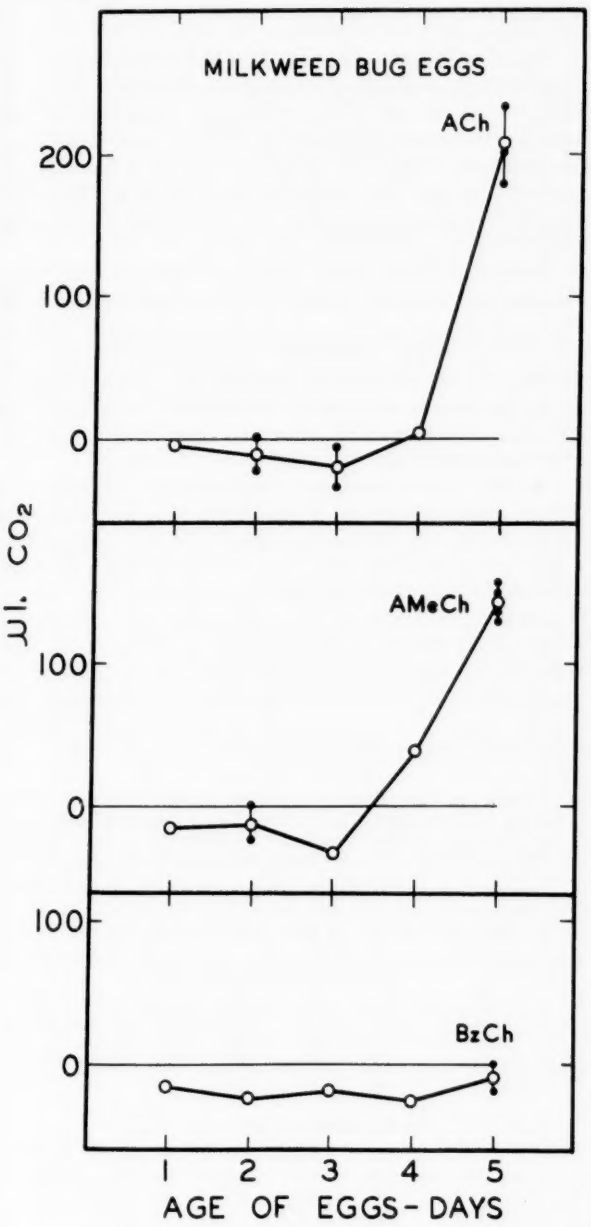

FIG. 2.—The occurrence of ChE activity in large milkweed bug eggs of various ages with three substrates (1×10^{-2} M).

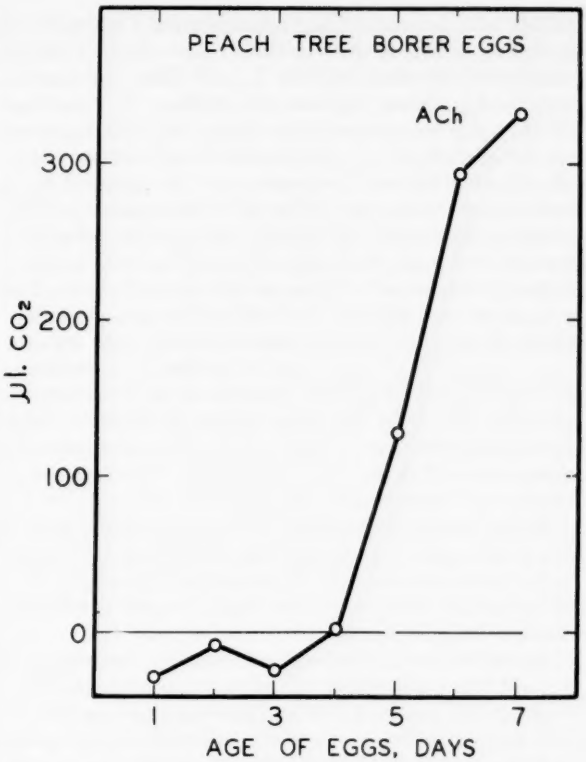

FIG. 3.—The occurrence of ChE activity in peach tree borer eggs of various ages using ACh (1×10^{-2} M) as substrate.

In both species activity first appeared about the fourth day and increased thereafter until hatching. Similar tests using other substrate concentrations were run and in all cases activity appeared at approximately the same time. With milkweed bug eggs the activity curves for ACh and AMECh were essentially the same shape, the difference being chiefly one of rate. At no stage of development was there appreciable activity with BzCh. It would appear from these data that while a quantitative change occurs during embryonic development of this species the properties of the esterases are rather constant.

In attempting to establish the relationship between ChE activity and susceptibility it is important to know whether the enzyme is totally absent or merely active at low levels. Such a distinction is not readily made by the technique employed here as allowances must be made for non-specific hydrolysis and the blanks used may not duplicate precisely the reactions occurring in the presence of all reactants. Both substrate and brei blanks were run and these values subtracted from the gross CO_2 production. Calculated in this manner, activity in early stages falls on or below the base line rather than slightly above as would be the case unless allowance was made for the values from these two sources. A puzzling situation occurred when parathion was added to breis which presumably showed no ChE activity. Slight depression in CO_2 production occurred, forcing the curve still further below the base line. The reason for this response is not clear but it implies that parathion had a slight depressing effect on non-specific hydrolysis. These findings on occurrence of ChE agree essentially with those of Chino & Yushima

(1954) who determined the appearance of ChE activity in eggs of the rice stem borer *Chilo simplex* Butl., cabbage armyworm *Baratha brassicae* L. and silkworm *Bombyx mori* L. Of interest also are the findings of Tahmisian (1943) using grasshopper eggs. Here ChE first appeared on the seventh day of pre-diapause and increased thereafter until diapause. Diapause was characterized by a uniform rate of activity followed by an increase in post-diapause. Lord & Potter (1954) working with extracts of tomato moth eggs demonstrated hydrolysis of ACh in late stages of development. These authors stress however that general esterase activity occurred at all stages of development. It appears from the investigations cited and the work reported here that in eggs of six species representing three orders of insects the pattern of ChE activity is broadly similar. In two cases (Chino & Yushima 1954, Tahmisian 1943) the methods employed were apparently more sensitive at low levels of activity than the manometric technique used by the writers.

It is of particular interest in the current study that in the two species considered, ChE activity occurred only in the latter half of the embryonic period. The significance of this point in relation to ovicidal susceptibility will be discussed later.

In vivo treatment.—Interest in *in vivo* treatment centered around two points: first, to determine whether ChE inhibition was involved in ovicidal action and secondly, to determine whether there was a differential in response between the susceptible and non-susceptible species with respect to ChE inhibition.

The effect of *in vivo* treatment on ChE activity is shown in table 2. It will be seen that for peach tree borer eggs inhibition was approximately 85% while in the milkweed bug inhibition was 15% or less. The exposures were above minimum lethal dosages for the peach tree borer as previously determined and sub-lethal for the milkweed bug. It would appear from these data that as in hatched forms lethal effect is related to a high degree of ChE inhibition. Inhibition of 67 to 85% for nerve cord AChE of adult cockroach at the prostrate stage of poisoning has been shown by Casida (1955) and almost complete inhibition of bee brain ChE at the point of death is reported by Metcalf & March (1949).

It has previously been demonstrated that eggs of the peach tree borer treated at all stages of development are susceptible to ovicidal action and that susceptibility is highest in the early stages (Smith 1955). The more recent finding that ChE is not present in early stages implies either that ChE is not involved in early treatments or that the toxicant is retained in the egg, later inhibiting ChE when it appears. As results just reported for peach tree borer eggs (table 2) involved exposures which may have overlapped the initial appearance of ChE activity, another test was conducted in which exposure was limited to the 24 hours following deposition and ChE activity determined on the fifth day. The results of this test are as follows:

ChE Activity—b_{180} (Gross)		Per Cent Inhibition
Normal	Treated	
134	58	57

(Substrate: 3×10^{-2} M ACh).

Over 50% inhibition occurred from early exposure. While this is somewhat less than the inhibition shown in table 2, the results are not directly comparable as the gross activity was considerably lower in early exposures and different substrate concentrations were used. It appears, however, that inhibition of ChE takes place as a result of exposures made before its occurrence, suggesting that the toxicant is retained in the egg.

In vitro treatment.—A striking difference has been shown both in susceptibility of the two species to ovicidal action and in properties of ChE. The relationship between *in vivo* and *in vitro* treatment is of interest in attempting to account for differential susceptibility. Accordingly, *in vitro* treatments were made to establish 50% inhibition (IN_{50}) value and to compare the rates at which inhibition occurred. These data are shown in table 3.

At the higher concentrations inhibition occurred at a more rapid rate in peach tree borer eggs but with both species little change occurred after 3 hours. Consequently, most comparisons are based on readings at this time. Maximum inhibition was of the same order in both species with milkweed bug eggs showing no increase in inhibition with excess parathion. The IN_{50} values derived from these data (table 3) are approximately the same for both species being of the order of 3×10^{-3}M. These values appear high particularly for the susceptible species. However, speculation on the relationship between *in vivo* and *in vitro* treat-

Table 2.—Inhibition of ChE in eggs of the peach tree borer and large milkweed bug by *in vivo* treatment with parathion. Substrate: 1×10^{-2} M ACh.

Species	Age of Eggs (Days)	ChE Activity, b_{180} (Gross) Untreated	ChE Activity, b_{180} (Gross) Treated	Per Cent Inhibition
Peach tree borer	7	405	63	84
	6	394	58	85
Milkweed bug	5	266	227	15
	4	59	54	8

Table 3.—The effect of parathion concentration on inhibition of ChE in eggs of the peach tree borer and large milkweed bug by *in vitro* treatment. Substrate: 1×10^{-2} M ACh.

Parathion Concentration (M)	Per Cent Inhibition of CO_2 Production 1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
	5-day-old Milkweed Bug Eggs					
1×10^{-1}	64	76	81	82	86	85
1×10^{-2}	69	78	81	83	86	85
1×10^{-3}	50	62	65	67	69	67
1×10^{-4}	29	29	31	32	32	27
1×10^{-5}	29	17	13	13	10	4
	6-day-old Peach Tree Borer Eggs					
1×10^{-2}	80	86	90	89		
1×10^{-3}	71	80	83	81		
1×10^{-4}	20	29	31	25		
1×10^{-5}	10	9	7	4		
1×10^{-6}	14	11	9	11		
1×10^{-7}	9	8	2	4		

Table 4.—The inhibition of ChE in eggs of the peach tree borer and large milkweed bug by various treatments. Substrate: 1×10^{-2} M ACh.

Treatment	Per Cent Inhibition	
	Peach Tree Borer	Milkweed Bug
In vivo	85	16
In vitro[a]	85	65
In vivo+*In vitro*[a]	87	66

[a] Parathion 1×10^{-8} M.

ment is limited by lack of specific knowledge as to whether the inhibitor is parathion or metabolites of parathion.

The similarity in IN_{50} value for the two species by *in vitro* treatment is in contrast to the marked differences by *in vivo* treatment. This could hardly be explained on the basis of diverse properties of ChE present as such differences would likely be manifest in both methods of treatment. It seems more likely that esterases from both sources are sensitive to inhibition to a considerable degree but in the case of the milkweed bug egg the toxicant fails to reach the site of action. The fact that a threshold of toxicity was not reached by *in vivo* treatment despite the use of excessive concentrations further supports this possibility.

While essentially the same degree of inhibition occurred in borer eggs by both methods of treatment, it was not known that the hydrolysis occurring in both cases was due to the same esterases. If the remaining activity was due to hydrolysis by the same esterases in both treatments then no further inhibition would result if *in vivo* treated breis were further treated *in vitro*. Tests of this type were conducted with breis from both species and the results are shown in table 4.

Double treatment resulted in no increase in inhibition over that achieved by *in vitro* treatment alone. Apparently the hydrolysis occurring *in vivo* and *in vitro* is due to the same esterase activity and the hydrolysis taking place with double treatment is due to esterases insensitive to parathion inhibition.

Further tests were conducted with milkweed bug eggs of various ages to compare inhibition rates using both ACh and AMeCh as substrates. These results are shown in table 5. The degree of inhibition was essentially the same, the chief difference being in the rate of hydrolysis with the two substrates. It is interesting to note that in eggs which presumably had no ChE activity (1- and 2-day) the addition of parathion resulted in depressed CO_2 production. While the total activity is too small to permit accurate comparison the tendency toward depression by parathion is rather consistent. Such a difference, if real, might be indicative of slight esterase activity although this seems unlikely considering the results previously reported on occurrence of ChE in developing eggs.

Table 5.—*In vitro* inhibition of ChE in eggs of the large milkweed bug by parathion. Substrate concentration 1×10^{-2} M; parathion concentration 1×10^{-3} M.

Age of Eggs (Days)	ACh, b_{150}		Per Cent Inhibition, 3 Hr.	AMeCh, b_{150}		Per Cent Inhibition, 3 Hr.
	Brei	Brei+ Parathion		Brei	Brei+ Parathion	
5	273	105	62	234	83	64
5	284	92	68	211	68	68
4	55	45	18	107	46	57
2	37	18	51	51	31	49
1	38	19	50	54	29	46

Discussion.—ChE activity occurred about the fourth day in eggs of the peach tree borer which required 8.5 days for completion of incubation at 80° F. In milkweed bug eggs having an incubation period of 6.1 days ChE activity occurred about the fourth day also. In both species activity increased progressively from the fourth day until hatching. The level of ChE activity for peach tree borer eggs was about three times that of milkweed bug eggs on a weight basis.

In vivo treatment resulted in marked ChE inhibition in the susceptible species and little inhibition in the nonsusceptible species. By contrast, *in vitro* treatment gave over 50% inhibition in both species. The properties of ChE from the two sources were found to differ widely.

In vivo treatments of the susceptible species before or after the appearance of ChE activity ultimately resulted in marked inhibition of the enzyme. This is considered evidence that as in hatched forms ChE inhibition is involved in toxic action. In treatments made before the appearance of ChE the toxicant is apparently retained in the egg, later acting upon the enzyme.

It has been suggested by Lord & Potter (1954) and others that in organisms where the nervous system is not developed and ChE systems are not physiologically active, the inhibition of enzymes hydrolyzing other acetate esters may be the primary mechanism of poisoning. Furthermore, activity by other esterases before the occurrence of ChE has been demonstrated by these workers. Their view regarding the role of other esterases was supported in part by the observation that development was arrested in eggs treated with TEPP before ChE appeared. The relationship of this evidence to inhibition as it normally occurs is doubtful as the concentration used was greatly in excess of practical concentrations and may have introduced modes of action which do not occur under conditions of actual usage. By contrast, dosages eight times the concentration employed in practice failed to halt development of peach tree borer eggs (Smith 1955). Of interest in this connection are the findings of Casida (1955) using organ systems of hatched forms. In tests involving eight organ systems and four inhibitors the only enzyme consistently inhibited to a large extent at the prostrate stage was AChE of the nerve cord.

The correlation between ovicidal effectiveness and *in vivo* inhibition by parathion for the susceptible and nonsusceptible species is in keeping with the concept of ChE inhibition as the mechanism of poisoning. The difference in susceptibility could conceivably be accounted for by the diverse properties of the ChE of the two species. A parallel has been cited by Metcalf & March (1950) in accounting for differences in susceptibility of flies and bees to certain organophosphates. If properties of ChE accounted for differences in susceptibility of the two species a corresponding difference in inhibition by *in vitro* treatment would be expected although evidence on the correlation between *in vitro* inhibition and insecticidal effectiveness is not in complete agreement. The work just cited shows a high correlation while Lord & Potter (1954) were

unable to find any correlation. The latter workers claimed that parathion was not an inhibitor of ChE *in vitro* requiring *in vivo* conversion before becoming an inhibitor. While this likely occurs in some cases the reverse was true with milkweed bug eggs, extensive inhibition occurring only *in vitro*. This could more logically be accounted for by failure of the toxicant to reach the site of action than to insensitivity of the ChE present. Further evidence on this point is provided by the observation that when eggs exposed to treated filter paper hatched, the nymphs died upon exposure to the treated surface. Thus, a lethal threshold by ovicidal action was not reached although toxicity to hatched forms could readily be demonstrated. In making such a comparison it would be helpful to know the LD_{50} value for newly hatched nymphs.

It has been postulated that the toxicant gains entry in the vapor form, this being predicated on the fact that direct contact between the egg and toxic residue is not required for ovicidal action. It is also evident that once exposed the toxicant is retained in the egg. In view of the foregoing, the ineffectiveness of parathion in the case of the milkweed bug egg might be explained by exclusion of the toxicant due to the characteristics of the chorion, or failure of the toxicant to become fixed and thus accumulate after gaining entry through the chorion. Such factors likely operate independently of the matter of enzyme sensitivity in which the properties of the cholinesterases play a role. In considering the permeability of the chorion the extensive investigations of Salkeld & Potter (1953) are of interest. Here permeability was found to vary with respect to species and stage of development. These workers also suggested that in some cases differences in susceptibility to Lepidoptera and Hemiptera may be due to differences in the method of hatching. Hemiptera are freed by splitting the chorion while Lepidoptera eat their way out and in so doing are likely to be exposed to or to ingest toxic substances. Such an explanation hardly seems applicable here as vital physiological systems are known to be affected before the developing larvae attempt to emerge from the chorion. Furthermore, when seemingly mature embryos are artifically removed from treated eggs they are incapable of coordinated activity and soon die.

It is striking that under practical conditions parathion residue appears to exert ovicidal effectiveness at levels which are not effective against hatched forms. If the toxicant is accumulated throughout the extended incubation period lethal levels might build up before hatching whereas such build-up seems unlikely in hatched forms. On the other hand, it has been demonstrated that exposures of several hours are sufficient to produce ovicidal action under laboratory conditions.

The findings presented here add validity to the hypothesis previously advanced to explain ovicidal action. In the susceptible species ovicidal action was associated with inhibition of ChE. When eggs were treated in early stages of development the toxicant was apparently retained in the egg until the appearance of the enzyme. In the nonsusceptible species, the properties of ChE were found to differ from those of the susceptible species but there was no evidence of insensitivity to inhibition by *in vitro* treatment. It seems more likely that ineffectiveness was due primarily to failure of the toxicant to enter the egg and reach the site of action although differences in susceptibility of the respective cholinesterases may exist.

SUMMARY AND CONCLUSIONS.—In eggs of the peach tree borer *Sanninoidea exitiosa* (Say) and large milkweed bug *Oncopeltus fasciatus* (Dall.) having incubation periods of 8.5 and 6.1 days, respectively (80° F.), ChE activity occurred about the fourth day and increased thereafter until hatching.

ChE activity in eggs in advanced stages of development was approximately three times as great for peach tree borer eggs as for milkweed bug eggs.

In ovicidal action of parathion to eggs of the peach tree borer, cholinesterase inhibition occurred regardless of whether treatment was made before or after its occurrence. This evidence is believed to implicate cholinesterase inhibition with ovicidal action and to demonstrate the retention of the toxicant by treated eggs.

The properties of esterases from the two species were found to differ but the relationship of this fact to ovicidal susceptibility was not established.

In vivo inhibition was associated with ovicidal effectiveness while *in vitro* inhibition caused over 50% inhibition in both susceptible and non-susceptible species.

The evidence obtained in the present study tends to support the scheme previously advanced that the toxicant taken up at any stage remains in the egg inhibiting cholinesterase when it appears and inhibition does not prove lethal until late stages of embryonic development.

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Late-Season Control of the Boll Weevil and the Bollworm With New Insecticides in 1955[1]

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Heavy late-season infestations of the boll weevil, *Anthonomus grandis* (Boh.) and the bollworm, *Heliothis zea* (Boddie), in central Texas in 1955 afforded an opportunity to conduct field experiments with several new insecticides for the control of these insects. The boll weevil was of primary importance in five experiments and the bollworm in three. Where the bollworm was involved in the boll weevil experiments, DDT was added to the insecticides and was used in the checks so that the bollworm had little, if any, influence on the results. At the time the bollworm experiments were conducted, boll weevil infestations were too low to appreciably affect results.

PROCEDURE.—Five experiments were conducted in small plots and three in large plots. In experiment 1 the plots were 16 rows wide and of sufficient length for an area of 0.2 acre; those in experiments 2, 3, 6, and 7 were 12 rows wide and 0.1 acre in size. The plots were arranged in randomized blocks with four replications of each treatment. In the large-scale experiments, 4, 5, and 8, there were no replications of treatments.

In the small-plot experiments emulsions were applied with a 4-row, tricycle-type, self-propelled machine constructed especially for spraying small areas; an engine mounted on the frame propelled the machine and drove the pump. The total volume of spray applied was 7.5 gallons per acre. In the large-plot experiments emulsions were applied with 8-row, tractor-attachment, low-pressure, and low-volume spray machines at the rate of 6 gallons per acre. Three nozzles per row were used and the sprays were applied at a pressure of 60 pounds per square inch in all experiments. Dusts were applied with rotary hand guns. Applications were made at approximately 5-day intervals with two exceptions; 9 days elapsed between the sixth and seventh applications in experiments 1 and 4 because of rains.

The procedure and method of recording data were the same as those reported by Ewing & Parencia (1943). The cotton fleahopper, *Psallus seriatus* (Reut.), and spider mites, Tetranychidae, did not cause appreciable damage in any of the experiments. An infestation of the cotton aphid, *Aphis gossypii* Glov., built up to near injurious numbers in the untreated check and dieldrin plots in experiment 1, but was controlled on September 1 with demeton before damage occurred. An infestation also occurred in the dieldrin-DDT plot in experiment 4, but it was not severe enough to warrant control. Infestations of the cotton leafworm, *Alabama argillacea* (Hbn.), in late September defoliated plants in untreated checks in all boll weevil experiments except experiment 3. Since it was late in the season and weevil infestations were very heavy, yields were not affected. Maturity of plants and residual control prevented defoliation in the treated plots, although there was some ragging of top growth in those in which boll weevil control was poor.

The treatments used in these experiments are given in table 1. The toxaphene and BHC-DDT-sulfur and Endrin dusts and the toxaphene and dieldrin-DDT mixtures and endrin sprays were commercial products. The other dusts and emulsifiable concentrates were obtained from various manufacturers.

RESULTS AGAINST BOLL WEEVIL.—In *Experiment 1* each treatment reduced the boll weevil infestation significantly below that of the check, and the 0.25- and 0.375-pound dosages of Bayer 17147 gave significantly better control than dieldrin, Bayer 16259, or 0.125 pound of Bayer 17147. The standard, dieldrin, was used at too low dosages early in the experiment for the infestation involved. The first two applications were made at 0.25 pound, the next three at 0.375 pound, and the last four at 0.5 pound, for an average of 0.4 pound per acre. One pound of DDT was added to all the insecticides for bollworm control. The check plots were also treated with 1 pound of DDT on five dates. The dieldrin and check

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[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Effectiveness of several organic insecticides against the boll weevil and the bollworm in field plot experiments.

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Active Ingredient per Acre (Pounds)	Per Cent of—			Seed Cotton (Pounds per Acre)	
		Boll Weevil Punctured Squares	Bollworm Injured			
			Squares	Bolls	Total	Gain Over Check
Small-Plot Experiments[a]						
Experiment 1 (9 spray applications between July 20 and September 1)						
Dieldrin, 1.5	0.4	52.9	4.7	2.7	1730	1417
Bayer 17147, 2	.125	46.4	6.2	3.2	1708	1395
	.25	32.4	5.0	1.4	2070	1757
	.375	27.5	4.5	1.5	2074	1761
Bayer 16259, 2	.25	49.2	5.2	2.7	1865	1552
Untreated	—	78.3	9.3	5.8	313	
L.S.D. at 5% level		11.6	2.3	1.6	219	
Experiment 2 (11 spray applications between July 20 and September 7)						
Chlorthion, 4	0.68	64.1	4.7	2.0	1672	710
Bayer L 13/59, 50[b]	.68	74.4	4.1	2.5	1073	111
Bayer 17147, 2	.25	34.2	4.5	2.2	2318	1356
Strobane, 8	2.7	61.7	4.3	2.6	1640	678
Toxaphene, 6	2.7	56.0	4.7	1.9	1920	958
Untreated	—	78.8	5.3	2.0	962	
L.S.D. at 5% level		7.1	—	—	239	
Experiment 3 (5 applications between July 8 and 28)						
Sprays						
DDVP, 0.5	0.4	60.5	4.1	4.3	421	−11
DDVP, 0.5+Aroclor, 2	.4+1.6	60.6	4.0	4.9	455	23
Dusts[c]						
Toxaphene 20+sulfur, 40	2.9+5.8	42.6	4.0	3.3	662	230
DDVP, 2.5	0.41	67.0	3.0	5.2	432	0
DDVP, 2.5+Aroclor, 10	.44+1.76	61.5	4.0	5.6	476	44
Untreated	—	65.9	3.8	4.6	432	
L.S.D. at 5% level		8.0	—	—	62	
Experiment 6 (3 applications between July 13 and 22)						
Dusts[d]						
BHC gamma, 3+DDT, 5+sulfur, 40	0.48+0.8+ 6.4	22.5	5.5	7.7	1324	171
Endrin, 2	0.33	12.5	3.0	6.7	1385	232
Bayer 17147, 5	.58	14.4	6.4	9.7	1387	234
Sprays						
Endrin, 1.6	.3	11.7	4.2	8.3	1424	271
Bayer 17147, 2	.5	15.5	6.2	13.4	1159	6
Untreated	—	23.4	8.1	19.1	1153	
L.S.D. at 5% level		—	3.0	4.0	111	
Experiment 7 (4 spray applications between July 14 and 27)						
Toxaphene, 4+DDT, 2	2.0+1.0	14.3	5.5	9.5	1455	230
Bayer 17147, 2	0.375	9.9	8.0	17.3	1284	59
	.5	12.2	8.2	12.8	1379	154
	.625	9.0	7.8	12.1	1411	186
Bayer 17147, 2+DDT, 2	.375+0.5	11.0	8.2	11.5	1421	196
Untreated	—	17.3	13.0	18.9	1225	
L.S.D. at 5% level		5.0	3.3	3.5	116	
Large-Plot Experiments[e]						
Experiment 4 (9 spray applications between July 20 and September 2)						
Dieldrin, 1+DDT, 2	0.43+0.94	39.1	6.1	4.1	2250	1989
Bayer 17147, 2+DDT, 2	.36+1.0	40.8	4.9	3.3	2415	2154
Untreated	—	88.9	2.2	1.8	261	
Experiment 5 (12 spray applications between July 28 and September 19)						
Dieldrin, 1+DDT, 2	0.375+0.75	48.8	1.5	0.3	621	620
Bayer 17147, 2+DDT, 2	.375+ .5	41.9	1.7	0.6	670	669
	.25 + .75	49.0	2.0	0.3	615	614
DDVP, 2.5+DDT, 2	.71+ .96	86.4	1.1	0.1	7	6
DDVP, 2.5+DDT, 2+Aroclor, 10	.71+ .96+ 2.84	84.6	1.1	0	6	5
Untreated	—	95.4	3.4	0	1	

Table 1—(Continued)

INSECTICIDE (POUNDS PER GALLON IN SPRAYS, PER CENT IN DUSTS)	ACTIVE INGREDIENT PER ACRE (POUNDS)	PER CENT OF—			SEED COTTON (POUNDS PER ACRE)	
		Boll Weevil Punctured Squares	Bollworm Injured			
			Squares	Bolls	Total	Gain Over Check
Experiment 8 (4 spray applications between July 14 and 27)						
Toxaphene, 4+DDT, 2	2.0+1.0	12.5	0.8	7.0	1903	417
Endrin, 1.6	0.3	15.5	2.0	6.2	1974	488
Strobane, 8+DDT, 2	2.0+1.0	18.2	3.3	6.3	1907	421
Bayer 17147, 2+DDT, 2	0.375+0.5	17.0	1.2	4.7	1853	367
Untreated	—	25.6	2.5	9.9	1486	

[a] Sprays applied at 7.5 gallons per acre.
[b] 50% soluble powder.
[c] Dust applied at 14.5 to 17.6 pounds per acre.
[d] Dusts applied at 11.6 to 16.5 pounds per acre.
[e] Sprays applied at 6 gallons per acre.

plots were treated with 0.25 pound of demeton per acre on September 1 for aphid control. Each treatment produced a significantly higher yield than the check. The two higher dosages of Bayer 17147 gave significantly higher yields than the low dosage or than dieldrin.

In *experiment 2* it soon became apparent that much better boll weevil control would be obtained with Bayer 17147 at 0.25 pound per acre than with the initial dosages of 2 pounds for toxaphene and Strobane and 0.5 pound for Bayer L 13/59 and Chlorthion. Consquently, the dosages of these insecticides were increased by 50% after the third application. Each insecticide except L 13/59 reduced the infestation significantly below that of the check. Bayer 17147 gave better control than any of the others. Toxaphene was better than Chlorthion and L 13/59, and Strobane and Chlorthion were better than L 13/59. The entire experiment had 1 pound of DDT per acre applied for bollworm control on the first nine application dates. Each insecticide except L 13/59 produced significantly more seed cotton per acre than the check. Bayer 17147 gave a higher yield than any of the other insecticides and toxaphene higher than Strobane or Chlorthion.

In *experiment 3* the toxaphene-sulfur dust gave significantly better control than DDVP dusts or sprays, with or without Aroclor (a chlorinated terphenyl). The DDVP treatments at approximately 0.4 pound per acre were no better than the check. Toxaphene-sulfur gave a higher yield than the other insecticides and check.

In large-plot *experiment 4* there was no difference in control between 0.43 pound of dieldrin and 0.36 pound of Bayer 17147. Better control was obtained with dieldrin than in experiment 1, which was in the same field, because the dosage was increased sooner. The respective gains in yield over the check were 1989 and 2154 pounds of seed cotton per acre.

Experiment 5 was conducted on poorer land and under deficient moisture conditions. The higher dosage of Bayer 17147 gave somewhat better control than the lower dosage or dieldrin. DDVP at 0.71 pound per acre, with or without Aroclor, failed to give appreciable control. Although yields were low, both dosages of Bayer 17147 and dieldrin gave good increases in yield, since the check and DDVP treatments produced practically no cotton.

RESULTS AGAINST BOLLWORMS.—In *experiment 6* each insecticide reduced the boll injury below that of the check. The BHC-DDT-sulfur dust and the endrin dust and spray gave better control than the 0.5-pound spray dosage of Bayer 17147. BHC-DDT-sulfur dust, Bayer 17147 dust at 0.58 pound per acre, and endrin dust and spray gave significantly higher yields than the 0.5-pound spray dosage of 17147 and the untreated check.

In *experiment 7* each of the other treatments gave better bollworm control than the 0.375-pound dosage of Bayer 17147. Toxaphene plus DDT, the 0.5- and 0.625-pound dosages of 17147, and 17147 plus DDT gave higher yields than the check. Each of these treatments, except the 0.5-pound dosage of 17147, gave a significantly higher yield than the 0.375-pound dosage of this insecticide.

In large-scale *experiment 8* good bollworm control and increases in yield were obtained with toxaphene plus DDT, endrin, Strobane plus DDT, and Bayer 17147 plus DDT.

SUMMARY.—Small-plot and large-scale experiments with several insecticides applied as dusts and as low-volume sprays were conducted in central Texas during 1955 for the control of late-season infestations of the boll weevil, *Anthonomus grandis* (Boh.), and the bollworm, *Heliothiszea* (Boddie).

In several experiments Bayer 17147 gave excellent boll weevil control at 0.25 and 0.375 pound per acre. In one experiment 0.125 pound of 17147 and 0.25 pound of Bayer 16259 were less effective. In another experiment Strobane and Chlorthion were less effective than toxaphene and Bayer L 13/59 was ineffective. Dieldrin at 0.43 pound per acre compared favorably in control and yield with Bayer 17147 at 0.36 pound in a large-plot experiment. DDVP dusts and sprays, with or without Aroclor, were ineffective at the dosages used.

Bayer 17147 at 0.375 pound per acre was ineffective against the bollworm, and at 0.5 pound it was less effective than recommended insecticides. A spray containing Bayer 17147 at 0.375 pound plus DDT at 0.5 pound per acre showed promise in this control.

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The Introduction of Mosquitoes of the Genus Toxorhynchites into American Samoa[1]

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The purpose of this paper is to place on record an account of the introduction of the giant predatory mosquitoes, *Toxorhynchites brevipalpus* Theobald and *T. splendens* Wiedeman, into American Samoa. The importation and establishment of these mosquitoes would, it was believed, aid in the control of the forest day mosquito, *Aedes pseudoscutellaris* Theobald, the principal vector of filariasis in American Samoa.

The term "filariasis" is an embracing one. Literally, it means infestation with filarial worms. In American Samoa the filarial worm prevalent is *Wuchereria bancrofti* (nonperiodic type). Persons suffering from filariasis usually manifest the disease in one or more of the following forms: filarial fever (fever with rigors), filarial lymphangitis, filarial abscesses, filarial glandular enlargement, filarial orchitis, elephantiasis. The disease may exhibit no symptoms for many years as many infected persons suffer little or no apparent ill effect throughout a large portion of their lives. The morbidity produced by the disease in the general population undoubtedly is high.

RELATIONSHIP OF *Aedes pseudoscutellaris* TO FILARIASIS. —*Aedes pseudoscutellaris* is a jungle- or bush-inhabiting mosquito which utilizes a multiplicity of small collections of water for the development of its aquatic stages. Any natural container such as a tree hole, rock hole, bamboo stump, or the leaf axils of such plants as pandanus, palms, taro, banana, ti, or ginger can serve as a suitable breeding site for *A. pseudoscutellaris*. The mosquito also breeds in empty coconut shells and the severed halves of seed pods of the cacao tree remaining from the harvesting of the seeds. Breeding occurs in artificial containers such as cans, bottles, auto tires, etc., but these sites are not so highly favored.

Aedes pseudoscutellaris breeds most extensively in densely vegetated areas such as the jungle and in plantation areas which are more or less overgrown with brush. The species also breeds extensively in coconut and cacao plantations where careless management permits the accumulation of empty shells and seed pods. Adult mosquitoes are rarely found resting in buildings, not even the thatched Samoan houses (fales), except when the latter are encompassed by the jungle. Even then, mosquitoes are seldom found resting in the fale although they may enter to feed upon the occupants. Resting mosquitoes are most often found on the underside of leaves, in the crevices of large rocks, rock walls, or rocky cliffs, and similar places in areas which are densely covered with trees or brush. Thus, the species seems to occupy primarily a natural or semi-natural ecological niche.

Aedes pseudoscutellaris is a day-biting species. Biting is more common during the early morning and late afternoon hours. That this habit is definitely linked to the incidence of filariasis is shown by Jachowski & Otto (1952). They point out that the Samoan in his native environment usually remains in his fale, or house during the heat of the day and after dark. He ordinarily is abroad tending his plantation or traveling from village to village during the cool morning hours and during the late afternoon. These periods of the day correspond with those during which the mosquito is most active. The microfilaria are most plentiful in the peripheral blood of infected individuals in the late afternoon. Thus, the habits of the Samoans, the preferred feeding times of the mosquitoes, and the characteristics of the disease combine to provide ideal conditions for the transmission of filariasis. Otto and Jachowski show that the index of potential transmission of filariasis is by far the greatest in the jungle, not in the Samoan villages.

The background of transmission data discussed above suggests that a program of mosquito control utilizing the usual methods of draining, filling, or chemical larviciding for the control of the aquatic stages of the mosquitoes, accompanied by fogging or residual spraying for the destruction of adult mosquitoes, might not be effective. Experimental attempts by the Naval Government of American Samoa to control *A. pseudoscutellaris* by utilizing orthodox means did not achieve the desired results. It thus became evident that some more feasible approach to the problem had to be found.

INTRODUCTIONS OF PREDATORY MOSQUITOES.—From time to time it was suggested that *Toxorhynchites brevipalpus* be introduced into American Samoa to aid in the control of *A. pseudoscutellaris*. This South African species was successfully introduced into Hawaii where a suitable technique was developed for mass rearing the mosquitoes (Bonnet & Hu 1952). The first introduction of *T. brevipalpus* into American Samoa was made in 1952 by D. H. Butchard. The species apparently did not become established as a result of this introduction. In January 1955 a second introduction of *T. brevipalpus* was made by C. P. Hoyt, then staff entomologist for the Department of Agriculture of American Samoa. At this time, also, the first introduction of *Toxorhynchites splendens* was made. Stock colonies of predatory mosquito larvae for each of these introductions were obtained from the Bureau of Mosquito Control, Department of Health, Honolulu, Hawaii. Hoyt established breeding colonies in the laboratory and ultimately liberated several hundred adult mosquitoes in various parts of the island of Tutuila. The breeding program was discontinued when Hoyt departed from American Samoa in May 1955.

In July 1955 the author was appointed staff entomologist for American Samoa and upon his arrival in the islands brought with him several hundred third and fourth stage larvae of *Toxorhynchites brevipalpus* and *T. splendens* to re-establish breeding colonies in the laboratory on Tutuila. This material likewise was obtained through the courtesy of the Bureau of Mosquito Control in Honolulu. Larvae of this shipment were transported in small glass novocaine vials. The earlier shipments were made utilizing small antibiotic bottles. There was less mortality in the

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[2] The author was staff entomologist for the America Samoan Department of Agriculture during the year 1955–56.

case of the antibiotic bottles. Breeding colonies were reestablished without difficulty and mass rearing of the giant predatory mosquitoes was resumed. Both *T. brevipalpus* and *T. splendens* utilize the same breeding sites as *A. pseudoscutellaris*.

MATERIALS AND METHODS.—The technique employed in mass rearing the predatory mosquitoes was essentially that developed by Bonnet and Hu. A number of problems arose during the course of the work, however, that resulted in the development of certain variations on their methods.

Breeding.—Breeding cages for the adult mosquitoes were 2 by 2 by 2 feet inside dimensions. The mosquitoes mated more readily in cages of this size and the cages provided ample room for breeding colonies of sizable numbers. Approximately 250 mosquitoes were maintained in each cage with both sexes represented in about equal numbers. Each cage was constructed of $\frac{3}{4}$- by $\frac{3}{4}$-inch wood frames covered with white organdie on the top and three sides; the bottom was a square of masonite. The rear side of the cage was covered with heavy unbleached muslin with a muslin sleeve 12 inches in diameter and 24 inches in length stitched to the center of the muslin panel to provide entry to the cage. The sleeve was tied off with heavy twine when not in use. At the start of the mass rearing program, the breeding cages were covered with fine mesh bronze screen. With the passage of time the screen became badly corroded due to the prevailing high humidity and the salt air from the nearby ocean and proved toxic to the resting mosquitoes. High mortality occurred in the cages until the source of the trouble was found. When the bronze screen was replaced with the organdie the excessive mortality terminated.

The adult mosquitoes were fed a mixture of 10% corn syrup in water. Early in the rearing program, balls of cotton dipped in the feed solution were suspended from the tops of the cages. This proved unsatisfactory due to the high rate of evaporation and the rapid development of mold. Petri dishes covered with a thin pad of cotton were substituted for the cotton balls. The pads were moistened with the feed solution periodically as necessary. This method proved very satisfactory. Some molding occurred, but this was easily checked by the simple expedient of turning the pads over each day for several successive days. Whether the cessation of mold development resulted from substances created during the slight fermentation that took place, or from salivary secretions of the mosquitoes during feeding is not known. The development of mold was rare, however, after the third or fourth day that a pad was placed in use. Water was provided in the same manner as the syrup-water mixture.

Sections of bamboo approximately 3 inches in diameter by 4 inches in depth and half filled with water were placed in each cage in which the females deposited their eggs. Four sections per cage sufficed. Observations on copulation and egg-laying were essentially the same as those previously reported by Bonnet and Hu. The females of both species of predatory mosquitoes deposit their eggs singly on the surface of the water. The eggs are small and white with a pinkish or buff tinge. *T. splendens* eggs are somewhat smaller than the head of an insect pin, whereas those of *T. brevipalpus* are slightly smaller than those of the former species. A small camel's-hair brush, such as used for water color painting, was used to pick up the eggs and transfer them to hatching pans. It was necessary to transfer the eggs each day, otherwise hatching occurred in the egg receptacles.

Rearing.—The *Toxorhynchites* eggs were hatched in enameled pans approximately 10 inches in diameter and 3 inches deep. Best results were obtained when the water in the pans did not exceed 1 inch in depth. A maximum of 200 eggs was placed in each pan. Egg rafts of *Culex annulirostris* Skuse, *C. quinquefasciatus* Say, or *C. sitiens* Wiedeman were placed in the pans to provide food for the newly hatched *Toxorhynchites* larvae. The *Toxorhynchites* eggs usually hatched within 36 hours. The same was true of the *Culex* eggs; therefore, the latter were placed in the pans immediately after the transfer of the *Toxorhynchites* eggs. It was noted that in nature the *Culex* eggs usually matured on the surface of the water in about 48 hours. If the eggs were disturbed, however, they hatched in a shorter period of time. This was always the case with egg rafts collected in the field and brought to the laboratory for feeding purposes. Fifty rafts per 200 *Toxorhynchites* eggs was about the proper ratio. Fewer egg rafts usually did not provide a sufficient number of *Culex* for the hatching *Toxorhynchites* larvae and a greater number sometimes overcrowded the pans upon hatching, resulting in excessive mortality. Water used in the hatching pans was stagnant water collected from the field and passed through a fine mesh sieve to remove leaves, sticks, clumps of algae, and similar debris. This water was rich in both inorganic and organic matter held in solution or in colloidal suspension. Water from the laboratory taps proved unsatisfactory because of the chlorine content and the absence of any material which could be utilized as food by the *Culex* larvae.

The *Toxorhynchites* larvae were allowed to remain in the hatching pans until they developed to the third instar. The young larvae fed voraciously, therefore concentrations of *Culex* larvae had to be high in order to prevent undue cannibalism among the *Toxorhynchites* larvae, which consumed about 10 *Culex* larvae each per day. During the first three instars it was necessary to replenish the supply of *Culex* larvae daily. In later stages of development, heavy feeding would carry the carnivorous larvae through a 48-hour period. Mature and nearly mature larvae were able to survive for long periods without food. Hoyt, for example, succeeded in keeping larvae alive for 28 days without feeding them. If abundant food was not provided during the early instars, however, mortality was high. It was observed that larvae which received an insufficient amount of food during the early stages of their development frequently died during transformation to the pupal stage or the adults failed to emerge.

The primary source of *Culex* egg rafts and larvae used in the rearing program was an abandoned, uncovered, concrete septic tank which was situated on a beach and partly surrounded by a dense thicket of mangrove, *Rhizophora* sp., and other tropical vegetation. The tank was approximately 12 by 20 feet and 4 feet deep and usually contained about 2 feet of water. There was a heavy growth of green algae in the tank. Adult female mosquitoes oviposited in the tank nightly, except during periods of inclement weather, and larvae occurred by many thousands. A collection of 5000 to 10,000 larvae per day, plus several hundred egg rafts, did not appear to reduce the population in

the tank in the slightest degree. During periods of heavy rain it was sometimes difficult to obtain sufficient egg rafts because the females were unable to oviposit, but larvae could usually be taken. During two particularly heavy storms the tank was completely flooded and all of the larvae washed away or destroyed. This effectually stopped breeding for some time and breeding operations at the laboratory had to be reduced to a bare maintenance level until the *Culex* population in the tank returned to its normal density. The most abundant species collected from the tank was *Culex sitiens* followed closely by *C. quinquefasciatus*. *C. annulirostris* larvae were always present, but in much smaller numbers. The *Toxorhynchites* larvae appeared to prefer the *sitiens* larvae and much better hatches were obtained from *sitiens* eggs than from those of either of the other species.

A large, shallow soup dipper and a fine mesh tea strainer were used to collect the *Culex* larvae. The larvae were skimmed from the surface of the water in the septic tank with the dipper and poured into the tea strainer. The strainer was then immersed at an oblique angle in a gallon can or jar filled with water from the tank and the larvae floated away from the strainer. Egg rafts were carefully dipped from the surface and placed in coffee cans which contained a suitable amount of water. In feeding the *Toxorhynchites* larvae it was found expedient to concentrate the *Culex* larvae in the strainer and while the latter was immersed in an upright position in a small bowl of water to draw the larvae into a small glass pipette fitted with a rubber suction bulb. The larvae were then dropped in measured quantities into the rearing containers which held the carnivorous larvae.

Toxorhynchites larvae became very pugnacious in the third instar and if they were allowed to remain together in large numbers many were killed. Consequently, third stage larvae were transferred to 2-ounce glass ointment jars—1 larva per jar—to complete their development. The jars were kept half filled with water. At this stage tap water was suitable. Actually, the carnivorous larvae required little water to develop. They would go through their larval stages and pupate in the small antibiotic bottles. The 2-ounce jars were more convenient to handle, however, and the larger quantity of water they contained did not become so foul from feces and other debris, thus providing a healthier environment for the larvae. During the latter part of the rearing program, the glass jars were discarded in favor of small cans such as are used in packing tuna. This was purely an economic measure. The glass jars were relatively expensive and easily broken, whereas the cans could be obtained in quantity at no cost from a local fish cannery. It was the practice of the cannery to open a number of cans each day for testing purposes; these used cans were turned over to the laboratory. The cans were as satisfactory in every respect as the glass jars.

The time required for the *Toxorhynchites* larvae to develop to the pupal stage could be shortened appreciably by exposing them constantly to electric lights. The majority of larvae which were not held under lights developed slowly to the last instar and then entered a semi-resting state during which feeding continued, but pupation did not occur. This resting state could be broken and the larvae made to transform by placing them under lights. An electrically wired rack, consisting of several open, ladder-like shelves was constructed for the rearing room. On this rack were placed wooden trays containing the rearing cans. Each tray held 36 cans and the rack accommodated 1080 cans. Each shelf was illuminated by means of 15-watt electric lamps spaced at proper intervals so that all the rearing cans were constantly under direct light. The trays were removable to facilitate feeding and handling of the larvae.

During the early part of the rearing program, the *Toxorhynchites* pupae were individually collected from the rearing jars and placed on wet cotton pads in tuna cans. A maximum of 25 pupae were placed in a can to avoid crowding. The cans were then placed in cages for emergence of the adult mosquitoes. The adults emerged as readily from pupae held on wet cotton as from those held in water with the additional advantages that there was less mortality and the mosquitoes usually were slightly larger and more robust. When large numbers of pupae were held in water, many of the emerging adults drowned through being pushed beneath the surface by others before they were entirely free of the pupal skin. When emergence began, food was placed in the cages. The time of development from hatching to adult mosquito averaged 17 days for both species.

The method of concentrating the *Toxorhynchites* pupae described above was tedious and time consuming. Therefore, experiments were conducted to develop a faster procedure. The technique eventually adopted was to pour the pupae from the rearing containers into a round sieve which was 8 inches in diameter and 2 inches deep with mesh openings 2 mm². This permitted both rapid concentration of the pupae and their swift separation from the water and accumulated debris of the rearing containers. The pupae were then placed on wet cotton mats in metal trays 8 by 12 inches—200 per tray. The trays were placed in emergence cages 12 by 18 by 18 inches constructed of $\frac{3}{4}$- by $\frac{3}{4}$-inch wood frames covered with white organdie. The bottoms of the cages were of masonite and were detachable to facilitate placing the pupae trays inside and also for the liberation of the adult mosquitoes. This method proved satisfactory in every respect and greatly reduced the time required to handle the pupae. The adult mosquitoes were held in the cages for about 5 days so that the females would be mated before they were liberated. The mosquitoes were released in forest areas near villages where there were numerous tree holes and other favored breeding sites.

Establishment.—Containers placed at or near the sites of release of the *Toxorhynchites* adults were utilized, in addition to natural breeding sites such as tree holes, leaf axils, etc., to determine initial establishment of the two species in nature. These containers consisted of empty coconut shells nailed to trees at or near the release points. The coconut shells were filled with water and *Culex* larvae and egg rafts were placed in them. Both species of predatory mosquitoes have been recovered from the field. It is too early, however, to form any definite conclusions as to what the ultimate effect may be on the population density of *A. pseudoscutellaris*.

SUMMARY.—The forest day mosquito, *Aedes pseudoscutellaris* Theobald, is the principal vector of filariasis in American Samoa. Experiments conducted during the period of Naval administration of American Samoa indi-

cated that *A. pseudoscutellaris* could not be controlled by orthodox methods. The giant predatory mosquitoes, *Toxorhynchites brevipalpus* Theobald and *T. splendens* Wiedeman, were introduced in the hope that they might effect a worthwhile degree of natural control of *A. pseudoscutellaris*. The predatory mosquitoes were mass-reared in the laboratory and many thousands were released on the island of Tutuila. Rearing methods and equipment used are discussed. Both species of predatory mosquitoes have been recovered from the field indicating that initial establishment has been obtained. It is too early to know the ultimate effect on *A. pseudoscutellaris*.

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Beet Yellows Virus Transmission by the Green Peach Aphid[1]

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Beet yellows is a virus disease of sugar beets which has been severe enough in the past to command the attention of a large number of workers in the British Isles and continental Europe. A rather thorough review of the literature of beet yellows and other virus diseases of sugar beets is presented in a recent Czechoslovakian monograph (Drachovská-Simanová 1955). While its recognition as a definitive disease occurring in the United States has come of late, it recently has been the subject of a number of papers (Coons 1952, McLean 1953, Bennett & Costa 1954). Since the virus is present in California, and since this State contributes a large share of the total tonnage of sugar beets annually produced in the United States, it was felt that a somewhat detailed study of the vector-virus relationships was worth-while, in spite of the fact that the subject has been studied by workers in other parts of the world, notably England (Watson 1940, 1946). A generalized comparative presentation of some of the results of the present work with those previously obtained with beet mosaic virus is being presented elsewhere (Sylvester 1956).

MATERIALS AND METHODS.—The original virus source was a plant obtained from J. S. McFarlane, of the U. S. Dept. Agric. Experiment Station, Salinas, California. This plant had been inoculated from field sources. At the same time this plant was acquired, a number of leaves were obtained from field infected plants both on the Experiment Stations grounds, and from nearby commercial plantings. Subinoculations by means of aphids were made from all these sources, and the most severe symptom types were selected as virus sources for the remainder of the work. Throughout the entire research program, subinoculated plants exhibiting initial vein etching symptoms have been selected for virus sources. Thus, the results reported herein are experimentally limited to a relatively severe strain of the virus, one which initially shows strong vein etching and later the yellowing and red-freckling type of symptom which has been well illustrated many times (Drachovská-Simanová 1955). Whether or not the results obtained are of general application to the various strains of the beet yellows virus is not known, but the author is unaware of qualitative differences in the vector-virus relationships of the various strains of the beet yellows virus and the green peach aphid, *Myzus persicae* (Sulz.).

The vector used was the green peach aphid, and the noninfective colonies were kept on sugar beet or smooth leaf mustard, *Brassica juncea* (Coss), plants. Most of the feedings, whether acquisition or test, were done using a microleaf cage with a nylon screen (silk stocking) over one end of a cellulose acetate cylinder, with the other end being closed by a cork. By using this technique, it was possible to account accurately for the insects before and after use, and also to confine the feedings to specific areas of the plants. In general, collection of aphids from stock colonies was accomplished by aspiration, using a long cellulose acetate centrifuge tube, the tip of which had been cut off and covered by a nylon screen. This screen acted as a resilient surface from which the aphids bounced during aspiration, and consequently mortality due to injury was negligible. It is evident from the literature that such nylon screened micro-cages are being developed in various parts of the world for use in vector studies (Kaloostian 1955, Legg 1955). A review of the development of cages of similar type has been made by Maramorosch (1951). One only has to try a few transmission experiments to realize their advantages over the more conventional method of aphid transferring using the camel's-hair brush and caging the plants.

When control of the numbers of aphids was desired, the aphids, collected by aspiration, were counted and loaded into the individual cages, using a camel's-hair brush.

The test plants were beets varying in size from the cotyledon stage to plants 3 to 4 inches high with 2 to 4 leaves. The variety was U. S. 15, and the seed was supplied by Mr. R. T. Johnson of the Spreckels Sugar Company, Spreckels, Calif. The seeds were germinated in vermiculite or sterilized sand, and transplanted into 3-inch clay pots filled with sterilized soil. If the test plants were under observation for 4 weeks or longer, they were watered once a week with ½ strength Hoagland's solution. Virus source plants were selected from subinoculations, and were transplanted into 6-inch clay pots. Weekly spraying with nicotine sulfate solution was done to reduce the chance of accidental infections in the greenhouse.

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ACQUISITION THRESHOLD PERIOD.—The work of Watson (1940, 1946) places the acquisition threshold period of beet yellows virus (Rothamsted strain) by the green peach aphid at approximately 5 minutes, with a maximum efficiency being obtained after approximately 6 hours of feeding on an infected plant. Bennett & Costa (1954), report the acquisition threshold period of beet yellows transmitted by the green peach aphid as being in the 5 to 10 minute range, with a maximum efficiency being obtained in 6 to 12 hours.

In the present study, the first series of replications, used U. S. 15 sugar beet seedlings, and late instar nymphs or green peach aphid apterae as the vectors, 1 aphid per plant, 10 plants per treatment, and 10 replications. The test feeding period had a mean value of 23.7 hours, with a range of 22.9 to 25.3 hours. The following access time intervals were used for the acquisition feeding: 0, 5, 10, 15, 30, 45, and 60 minutes. Out of a total of 595 plants tested, only three were infected, and all these were in the 60-minute interval.

In the second series of replications, again using single late instar nymphs or apterae of the green peach aphid, one aphid per test plant, these results were partially confirmed. In this series of tests using 10 replications, 10 plants per treatment, the access times used were: 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours. At the end of the access time, the insects were caged on seedling beets for a test feeding period of 24.03 ± 0.7 hours (mean plus or minus the standard deviation). A total of 6 different virus source plants were used, 4 for each of 2 replications, and 2 for 1 replication each. A variation of 2 to 29 plants were infected out of a 100 possible for each replication, but this could not be correlated directly with differences among the virus source plants. The treatment results (in ratios listed, numerator is number of plants infected; denominator, number tested; variations in denominators are due to test plants dying prior to symptom production) of the replications were as follows: check (0 access time), 0/93, 1 hour access time, 2/95; 2 hours, 7/94; 3 hours, 11/93; 4 hours, 9/97; 5 hours 11/98; 6 hours, 17/92; 7 hours, 21/97; 8 hours, 19/95; 12 hours, 24/97; 24 hours, 25/97. Again the results indicated that, under the conditions tested, the probability of aphids acquiring a virus charge in less than 1 hour of access time on a virus source was low.

An attempt to fit a regression line to the data (Sylvester 1956), indicated that one of the best curves obtained was that of a parabola, $Y = a + bX - cX^2$, with the value of $\hat{Y} = 14.23 + 3.34\ (X - 6.55) - 0.095\ (X^2 - 84)$. Statistical analysis indicated that the within treatment variation was such that deviations from either a linear or a parabolic regression line were not significant, but that the fit of the data was significantly better when using the parabola.

The implications of the parabolic fit are that initially there is a rise in virus acquisition as the access period is lengthened, but that eventually there is a gradual reduction in vector efficiency. The 24-hour access feeding was not sufficiently long to establish this point, but in subsequent work, aphids which had a single 24-hour access feeding were compared with aphids which had two 24-hour access feedings (total time, 48 hours), and it was found that there was a significant reduction in the efficiency of the 48-hour fed insects (Sylvester 1956). Hints of the same type of phenomena were obtained by Watson (1940) and were sufficiently noticeable to evoke comment. She suggested the advancing age of the insects was a possible explanation. The existence of such a phenomenon is suggestive of that which occurs in the transmission of nonpersistent viruses, with the exception that the time scales are vastly different, for in the nonpersistent viruses, the evidence of loss of infectivity can be detected in acquisition feedings of a minute or so, rather than in a period of hours, as is the case with beet yellows.

Differences in the minimum time for the acquisition threshold period in this work, and that of Watson (1940) and Bennett & Costa (1954), probably are associated with the level of vector efficiency.

INOCULATION THRESHOLD PERIOD.—Watson (1940) and Bennett & Costa (1954) report the inoculation threshold period for the beet yellows virus and the green peach aphid is between 5 and 10 minutes.

During 1953 and 1955 a series of tests were carried out to determine the inoculation threshold period of the beet yellows virus under the Berkeley conditions. In the 1953 tests, the aphids were allowed access to the virus source plants and then transferred by brush to the test plants which in turn were caged for the desired test feeding interval. In the 1955 work, the insects were allowed, except for one replication, an acquisition feeding period utilizing the leaf cage technique, and the test feeding periods were likewise done using leaf cages. In all experiments, the acquisition feeding period was 20.33 ± 3.43 hours in length, with the exception of one replication in which the insects were reared on the virus source.

The first series of tests in 1953 used 1 insect per plant, 20 plants per treatment, 7 replications, and the following test feeding intervals: 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours. Two of the replications were negative. The next series of 3 replications, 1 insect per plant, 20 plants per treatment, done in the fall of 1953, used the following test feeding intervals: 0, 1, 2, 3, 4, 8, and 24 hours. One replication was negative. In 1955, another series of tests were made, using 4 replications, 20 plants per treatment, 1 insect per plant, the leaf cage technique, and the following test feeding intervals: 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours. Two months later a final series was done, using 14 replications, 10 plants per treatment, leaf cage technique, 4 insects per cage, and the following test feeding intervals: 0, 1, 2, 3, 4, 5, 6, 8, and 24 hours. It was hoped that by increasing the number of insects per cage, a more valid estimation of the inoculation threshold period could be obtained.

Pooling the entire data for the replications in which transmission occurred, and the intervals of 0, 1, 2, 3, 4, 5, 6, 8, and 24 hours, the following results (plants infected/plants tested) were obtained: 0 hours (check), 0/359; 1 hour, 16/359; 2 hours, 20/357; 3 hours, 32/357; 4 hours, 28/357; 5 hours, 27/317; 6 hours, 35/317; 8 hours, 44/356; 24 hours, 39/355. Examination of the series for homogeneity using the chi square test, indicated a nonsignificant departure from homogeneity using the values obtained for 3, 4, 5, 6, 8, and 24 hours ($\chi^2 = 5.9$, d.f. 5, $p < 0.50$). However, when the results of the 2-hour period were put into the series, the chi square value was raised to 13.01, with 6 d.f., $p < 0.05$.

Using 11% transmission as the maximum obtainable

Table 1.—Observed and expected values for the transmission of the beet yellows virus by the green peach aphid, using variable test feeding intervals and the relationship $\hat{Y}=a(1-e^{-\alpha t})$.

TEST FEEDING IN HOURS	PER CENT TRANSMISSION (Y)	
	Observed	Expected[a]
1	4.45	3.63
2	5.60	6.05
3	8.96	7.70
4	7.84	8.80
5	8.51	9.51
6	11.00	10.01
8	12.35	10.56
24	10.98	11.00

[a] $\hat{Y}=11(1-e^{-0.4t})$.

under the experimental conditions, an exponential curve was estimated, using the relationship $\hat{Y}=a(1-e^{-\alpha t})$, $a=11\%$, $\alpha=0.4$, $t=$hours (Sylvester 1956). In table 1, the results are given for the observed and the expected per cent transmission of the beet yellows virus by the green peach aphids, using variable test feeding times, and the hypothesis that $\hat{Y}=a(1-e^{-\alpha t})$. Using chi square as a method of analysis with $n-2$ degrees of freedom, the sum of the 8 chi square values is 4.06, d.f. 6, $p>0.50$. Whether or not this type of evaluation is justified is not known, but it seems evident that the ability of the green peach aphid to inoculate beet plants with the yellow virus is not restricted to the same extent as is virus acquisition. Perhaps when virus is placed in a favorable tissue by the insect, it has a good chance to establish an infection, and that these tissues can be reached by the insects fairly rapidly. Feeding aphids for more than 6 hours did little to increase the chances of successful inoculation.

RETENTION.—The implied difference between nonpersistent and persistent aphid-borne viruses is the length of time which infective insects retain a change of virus sufficiently large enough to make inoculation during a test feeding period probable. Beet yellows was placed in the group of persistent aphid-borne viruses by Watson (1940), although she recognized that the period of retention was not of the same order as with other persistent aphid-borne viruses (Watson 1940, 1946). In fact, it was the relatively short time of persistency that lead Watson to suggest that retention was not a good criterion to use to classify insect-borne plant viruses into two groups, the persistent and the nonpersistent. In 1946 Watson (1946) was of the opinion that the essential difference between persistent and nonpersistent viruses was the influence of pre-acquisition feeding fasting on resulting vector efficiency. In the case of the nonpersistent viruses, this treatment increased vector efficiency (when coupled with a short acquisition feeding), while with persistent viruses, fasting prior to an acquisition feeding, had little or no effect on vector efficiency.

Watson (1940) found that beet yellows virus was retained by feeding aphids for periods of time not in excess of 72 hours, and Bennett & Costa (1954) reported that the virus can be retained by green peach aphids for 24 to 48 hours.

The following experiments were done to more clearly define the persistency of the beet yellows virus in feeding infective green peach aphids. In the first series of tests, using 122 lots of aphids, 10 aphids per lot (late instar nymphs and apterae, reared on the virus source plant), the insects were transferred daily to new test plants until the insects were dead. As the serial moves were made, insects died, and consequently the number of lots, and the number of insects per lot, tested each day decreased. The results obtained were as follows (ratios indicate number of plants infected out of number tested): 1st day, 68/115; 2nd day, 12/116; 3rd day, 1/114; 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, and 13th day, 0/114, 0/111, 0/75, 0/48, 0/30, 0/21, 0/11, 0/7, 0/3, and 0/1, respectively.

In a second test, using 110 lots of 10 aphids per lot, and daily serial transfers to healthy test plants for a maximum of 9 days, only 4 infections were obtained, and all occurred on the first day.

From these preliminary experiments, it was evident that the period of retention of beet yellows virus by green peach aphids was relatively short, and that if the rate of virus loss was to be determined more precisely, the serial transfer interval would have to be shortened. It was decided to use 8 hours as the interval of transfer, and the leaf cage technique to facilitate manipulation.

In the first experiment, 99 cages, 10 aphids (apterae and late instar nymphs) per cage, were used. The minimum acquisition feeding for the apterae was 1 week, and that for the nymphs was 48 hours. A total of seven, 8-hour transfers were made, with the following results: 1st period, 60/99 (plants infected/plants tested); 2nd period, 48/99; 3rd period, 26/99; 4th period, 16/99; 5th period, 15/99; 6th period, 9/99; 7th period, 5/99.

A second test was made, using 100 cages of aphids, 10 aphids per cage, and a total of 7 transfers at 8-hour intervals. The insects were reared on the virus source plants, and three sources were used. The following results were obtained: 1st period 48/100; 2nd period, 33/100; 3rd period, 9/100; 4th period, 4/100; 5th period, 1/100; 6th period, 1/100; 7th period, 0/100.

Both tests indicated that the period of retention of beet yellows virus by feeding infective green peach aphids was relatively brief, and that the rate of loss of infectivity was rapid and somewhat uniform.

In the first experiment, one virus source plant was used for cages 1 through 20; a composite sample from three virus source plants was used for cages 21 through 76 (with No. 52 missing); and a 5th virus source plant (a field infected large beet) was used for aphid lots No. 76 through 100. When the data were examined, it was found that the 5th virus source plant was infected with the beet yellow-net virus (Roland 1948, Sylvester 1949), in addition to the yellows virus. It was also noted that the percentage of transmission of beet yellows virus was much higher in this than in the other series. The results were: 1st period, 25/25; 2nd period, 20/25; 3rd period, 19/25; 4th period, 14/25; 5th period, 15/25; 6th period, 9/25; 7th period, 5/25. Thus there was evidence that insects carrying two viruses (yellows and yellow-net) might be more efficient at the transmission of beet yellows and could retain it longer, than insects carrying beet yellows virus alone.

The second experiment, done in an attempt to establish more firmly the length of the retention period of the yellows virus by aphids not carrying another virus, indi-

cated that most of the infective potential was gone by the end of the 4th 8-hour feeding period. Thus the possibility remained, that the yellow-net virus had a synergistic effect on the transmission and/or retention of the yellows virus by the green peach aphid.

In order to test the hypothesis that efficiency in acquisition and retention of the yellows virus by green peach aphids is not dependent on the presence or absence of the beet yellow-net virus, a relatively extensive series of experiments was done. A factorial design was used, employing all combinations of 2 feeding periods, 2 viruses (yellows and yellow-net) and 2 types of leaves on each virus source plant (leaves showing symptoms and leaves not showing symptoms). In addition, simultaneously, another factorial design comparison was made with plants infected with both yellows and yellow-net viruses (dual infection) comparing all combinations of two acquisition feeding periods on leaves with and without the symptoms of one or the other of the two viruses.

All experiments were done using the leaf cage technique. In experiment 1, transmission was accomplished using lots of 10 aphids per cage, 8 cages per virus source, and 5 plants for each virus source type (yellows, yellow-net, or dual infection). In experiments 2 and 3, lots of 5 aphids per cage were used, 8 cages per virus source, and 5 plants for each virus source type. In experiment 4, in replication 1, 5 plants, 8 cages per plant, of each virus source type were used; and in replications 2, 3, 4, and 5, single virus source plants were used, 8 cages per plant, 10 aphids per cage, with the aphids being divided into 10 cages, 1 aphid per cage, at the beginning of the test feeding sequence.

At the end of the acquisition feeding, the cages were attached to the first of the test plants in the series, and at the end of 8 hours, the cages were moved to the 2nd test plant series. This was repeated until a total of seven test plants had been used, 8 hours of feeding per test plant, for each cage of aphids. At the end of the test plant series the cages were removed and the number of aphids alive in each cage recorded. In the single insect experiment, whether or not the aphid was alive was noted at the end of each 8-hour period; and if the insect had died, transfers were discontinued, but the test plants that would have been used, were kept in the series to serve as checks on greenhouse spread of the virus. A total of 352 such check plants were in the series of replications, and no virus infection occurred.

It might appear that there was unnecessary replication of the experiment. The major problem encountered was contamination of the aphids that were raised on sugar beets with the yellow-net virus. In experiments 1, 2, and 3, using lots of 10, 5, and 5 aphids per cage, respectively, it was found that there was a certain amount of contamination with the yellow-net virus in the original source of supposedly noninfective aphids. In experiment 3, it was also found that the original source plant upon which the aphid colony was being maintained had yellows virus, so that a proportion of the insects used were contaminated with both viruses. Consequently, these three experiments were not completely satisfactory to use as a basis to judge whether or not the presence of the yellow-net virus in aphids affected their ability to transmit and/or retain the yellows virus. It was because of this persistent low level of contamination that the final series of replications was done. In this last experiment, the aphids used were obtained from colonies reared on mustard, and in this case no contamination with virus was evident. The undesirable feature of this experiment was the extremely low level of transmission of the yellow-net virus.

Table 2.—Results[a] on the transmission of sugar beet yellows and yellow-net viruses by green peach aphids fed on virus source leaves for 24 hours before being serially transferred at 8-hour intervals to a total of seven test plant beet seedlings.

Virus Source Leaf	Experiment Number			
	1	2	3	4
Yellows[b]				
leaf with symptoms	16/70	22/70	22/70	13/324
leaf without symptoms	24/70	25/70	20/70	38/327
Yellow-net				
leaf with symptoms	30/69	24/70	36/70	7/330
leaf without symptoms	29/70	28/70	39/70	3/312

[a] In experiment 1, lots of 10 insects were used; in experiments 2 and 3, lots of 5 insects were used; while in experiment 4, single insects were used. In ratios listed, numerator is number of plants infected; denominator, number tested.

[b] In experiments 1, 2, and 3, the original colonies of insects were found to carry some yellow-net virus; the level of contamination was 22/140, 24/140, and 17/140, respectively.

However, with the limitations which might be imposed by the contamination of the aphids in the first three experiments, and the low level of yellow-net transmission by individuals in the 4th experiment, it is believed that the data are worth presenting and evaluating to a certain extent. Analysis has been done on various levels. In the main, the results were analyzed with the assumption that the differences due to varying numbers of insects per test are differences in degree not kind. In some cases, however, the data have been reduced to the expectancy of transmission by single insects. The contamination factor was noted as being present, but no adjustment in the form of evaluating a correction factor was made. In some cases, an adjustment to account for the mortality of aphids within groups during serial transmission was made, and the data reduced to the expectancy of transmission by single insects. In these cases, linearity was assumed to establish the rate of death. When necessary, variations due to differences between individual virus source plants have been assessed in order to gain support for differences between treatment. In arriving at the conclusions presented, it is hoped that proper consideration of the various factors which contributed to data variability has been given.

In table 2, a comparison is made of the results of trials done to compare the transmission of the beet yellows virus, or the beet yellow-net virus from virus source leaves showing symptoms of the disease and those not showing symptoms. The results were calculated as percentages, transformed to angle values (angle = arc sin $\sqrt{\text{percentage}}$),[2] and subjected to analysis of variance. No significant difference in transmission due to the presence or absence of symptoms in the virus source leaves was found either in the case of the yellows virus or the yellow-net virus.

Chi square tests were run to compare the homogeneity of the various virus source plants as to the total amount of

[2] Values taken from tables by C. I. Bliss.

transmission of either yellows virus or the yellow-net virus from each of the five source plants used in experiments 1, 2, 3, and the eight virus source plants used in the replications in experiment 4. In experiment 1, the five source plants used for acquisition of the yellows virus were found to be homgeneous ($\chi^2=1.38$, d.f. 4, $p=>0.80$). In this experiment, the five plants used as virus source plants for the yellow-net virus were also found to be homogeneous ($\chi^2=2.36$, d.f. 4, $p=>0.70$). In experiment 2, the hypothesis of homogeneity likewise was not rejected for either the five plants used as sources for the yellows virus or the five used as sources for the yellow-net virus ($\chi^2=6.38$, d.f. 4, $p=>0.10$, and $\chi^2=5.13$, d.f. 4, $p=>0.20$, respectively). In experiment 3, there was a strong tendency for heterogeneity to exist among the five plants used as a source for the yellows virus ($\chi^2=12.56$, d.f. 4, $p=<0.02$), and also a heterogeneous tendency among the five plants used as sources for the yellow-net virus ($\chi^2=9.32$, d.f. 4, $p=0.05$ approx.). In experiment 4, the chi square test failed to support the hypothesis of homogeneity ($\chi^2=26.29$, d.f. 7, $p=<0.01$), but the low expectancy of transmission ($p=0.05$ expect transmission small), makes it difficult to judge the significance of the chi square value. However, it was felt that in general the variability due to the lack of homogeneity among all virus source plants in any one particular test was not of sufficient magnitude to invalidate the other conclusions.

In table 3 are given the results of the experiments designed to determine whether or not the presence of the yellow-net virus before or after the acquisition of yellows virus would influence the transmission of either of the viruses. Again the percentages of transmission were transformed to angles and the data subjected to analysis of variance. The following conclusions have some support (5% level of significance). The average transmission of the yellows virus was somewhat less than that of the yellow-net virus. Less virus transmission occurred if the insects began the feeding sequence on a yellows source than if they began the feeding sequence on the yellow-net source. Feeding combinations alone, *.i.e*, whether the feeding sequence was from leaves with symptoms to leaves with symptoms, leaves with symptoms to leaves without symptoms. etc., did not influence the total amount of virus transmission. There was less transmission of the yellows virus if the feeding sequence was begun on yellows infected source when compared to the transmission obtained when the feeding sequence was begun on a yellow-net source and terminated on a yellows source. Of more general interest was the second order interaction of virus×initial feeding (on yellows, or on yellow-net) ×feeding combination. For convenience, the results of this interaction are given in table 4.

From the table, it might be concluded that, in general, it is unfavorable for the transmission of the yellows virus for an aphid to leave a yellows virus source plant and feed on another plant, although the second plant can serve as a source for an additional virus. In the particular case in point, the second acquisition feeding on a plant not infected with yellows, merely served as a test feeding period for the insect, and the yellows virus was not maintained, but lost at a rate similar to that which would have occurred on healthy test plants.

Table 3.—Results[a] on the transmission of sugar beet yellows and yellow-net viruses by green peach aphids feeding on virus source leaves for two acquisition feeding periods and then being serially transferred at 8-hour intervals to a total of seven healthy test plants.

Original Virus Source	Second Virus Source	Virus Transmitted	Experiment Number			
			1	2	3	4
Yellows leaf with symptoms	Yellow-net leaf with symptoms					
		yellows	0/34	7/35	3/35	3/154
		yellow-net	22/34	10/35	13/35	1/154
with symptoms	without symptoms					
		yellows	0/35	6/35	7/35	2/145
		yellow-net	20/35	5/35	14/35	1/145
without symptoms	with symptoms					
		yellows	1/35	13/35	5/35	1/142
		yellow-net	21/35	10/35	21/35	1/142
without symptoms	without symptoms					
		yellows	0.35	12/35	9/35	4/164
		yellow-net	17/35	8/35	7/35	0/164
Yellow-net leaf with symptoms	Yellows leaf with symptoms					
		yellows	0/35	14/35	13/35	6/166
		yellow-net	5/35	14/35	20/35	2/166
with symptoms	without symptoms					
		yellows	4/35	21/35	12/35	8/132
		yellow-net	5/35	12/35	22/35	0/132
without symptoms	with symptoms					
		yellows	3/35	12/35	8/35	6/157
		yellow-net	4/35	11/35	18/35	0/157
without symptoms	without symptoms					
		yellows	8/35	14/35	8/35	28/194
		yellow-net	2/35	6/35	15/35	0/194

[a] In experiment 1, lots of 10 insects were used, and each acquisition feeding period was 24 hours in duration; in experiment 2 and 3, lots of five insects were used, with each acquisition feeding period being 12 hours in duration; while in experiment 4, single insects were used, with each acquisition feeding period being 12 hours in duration. In ratios listed, numerator is number of plants infected; denominator, number tested.

Table 4.—Mean values obtained from four experiments on the transmission of the sugar beet yellows and yellow-net viruses by green peach aphids[a] fed on virus source leaves for two acquisition feeding periods before being serially transferred at 8-hour intervals to a total of seven healthy test plants.

Original Virus Source (1st 12- or 24-Hour Feeding)	Second Virus Source (2nd 12- or 24-Hour Feeding)	Virus Transmitted	Angle[b]	Per Cent
Yellows leaf with symptoms	Yellow-net leaf with symptoms			
		yellows	9.95	3.0
		yellow-net	32.00	28.1
with symptoms	without symptoms			
		yellows	14.45	6.2
		yellow-net	28.82	23.2
without symptoms	with symptoms			
		yellows	18.59	10.2
		yellow-net	34.67	32.4
without symptoms	without symptoms			
		yellows	18.81	10.4
		yellow-net	24.41	17.1
Yellow-net leaf with symptoms	Yellows leaf with symptoms			
		yellows	24.38	17.0
		yellow-net	29.20	23.8
with symptoms	without symptoms			
		yellows	30.16	25.2
		yellow-net	27.64	21.5
without symptoms	with symptoms			
		yellows	23.18	15.5
		yellow-net	24.90	17.7
without symptoms	without symptoms			
		yellows	29.68	24.5
		yellow-net	19.79	11.5
L.S.D. 0.05			14.15	

[a] Lots of 10, 5, and single insects fed 12- or 24-hour periods.

[b] Angle = arc sin $\sqrt{\text{percentage}}$, values taken from tables of C. I. Bliss.

Of interest also is the tendency of the data to indicate that leaves with and without symptoms serve in different capacities as far as transmission of the two viruses is concerned. In general, it appears that leaves with symptoms tend to be better sources of the yellow-net virus than leaves without symptoms; while in the yellows virus, leaves without symptoms are better virus sources than leaves with symptoms. The results of this first order interaction are presented in table 5.

If the results of the analysis of variance are meaningful, it is evident that the transmission of yellows virus by green peach aphids tends to be best when leaves without symptoms are used, while the opposite is true of the yellow-net virus. It was the opposite effects on the transmission of the two viruses that was responsible for the nonsignificant variation in this experiment which was attributable to combination of feeding alone. Of further interest is the internal consistency of the data, for the type of leaf used for the second acquisition feeding influenced the results in the same manner as the total sequence, for the actual mean per cent transmission of yellows virus when the second acquisition feeding was on leaves without symptoms was greater than when that feeding occurred on leaves with symptoms. The opposite was true in the case of the yellow-net virus. The analysis of the results in table 1 does not support these conclusions, but perhaps the factorial design with a larger number of variables increased the sensitivity of the experiment to a point where the effects of source leaves and their symptom state could be measured with greater precision.

The next data to be considered are those obtained during the experiments in which the aphids were fed on plants infected with both viruses (dual infection). Within each experiment, the data were examined by the chi square test for homogeneity as to the ability of the various plants to serve as virus sources. In all experiments, it was found that the individual plants were not homogeneous as sources of either the yellows virus or the yellow-net virus, nor were the plants which were superior sources of one virus necessarily superior sources for the other virus when compared with each other within any one series.

The results obtained are given in table 6. Again the raw data were expressed as percentages, transformed into angle values, and subjected to analysis of variance. Significant variations were found to be due to viruses, yellows being transmitted in more cases than yellow-net. A significant amount of variation was found between experiments, but no significant variation could be attributed to the feeding combinations used. When concerned with individual plants which were infected with both the yellows and the yellow-net viruses, the amount of transmission of any single virus apparently was dependent upon factors other than whether or not the insects fed on leaves with or without the symptoms of the particular virus which was of immediate interest.

Table 5.—Mean values obtained from four experiments on the transmission of sugar beet yellows and yellow-net viruses by green peach aphids[a] fed on virus source leaves for two acquisition feeding periods before being serially transferred at 8-hour intervals to a total of seven healthy test plants.

Original Virus Source (1st Acquisition Feeding)	Second Virus Source (2nd Acquisition Feeding)	Virus Transmitted	Angle[b]	Per Cent
With symptoms	With symptoms			
		yellows	17.16	8.7
		yellow-net	30.60	25.9
With symptoms	Without symptoms			
		yellows	22.30	14.4
		yellow-net	28.22	22.4
Without symptoms	With symptoms			
		yellows	16.71	8.3
		yellow-net	29.79	24.7
Without symptoms	Without symptoms			
		yellows	24.24	17.1
		yellow-net	22.09	14.1
L.S.D. 0.05 for angle			7.07	

[a] Lots of 10, 5, and single insects fed 12- or 24-hour periods.

[b] Angle = arc sin $\sqrt{\text{percentage}}$, values taken from tables of C. I. Bliss.

Table 6.—Results[a] on the transmission of sugar beet yellows and yellow-net viruses by the green peach aphid fed on leaves of plants infected with both viruses and then being serially transferred at 8-hour intervals to a total of seven healthy test plants.

Symptoms of Virus Used as Source Leaf	Symptoms of Virus Used as Source Leaf		Experiment Number			
For Initial Acquisition Feeding	For Final Acquisition Feeding	Virus Transmitted	1	2	3	4
Yellows	Yellows	Yellows	4/35	25/70	32/70	26/338
		Yellow-net	12/35	19/70	12/70	4/338
Yellows	Yellow-net	Yellows	3/35	17/70	30/70	15/328
		Yellow-net	11/35	19/70	7/70	1/328
Yellow-net	Yellows	Yellows	3/35	24/70	29/70	25/323
		Yellow-net	16/35	30/70	9/70	0/323
Yellow-net	Yellow-net	Yellows	4/35	25/70	33/70	19/335
		Yellow-net	16/35	25/70	11/70	0/335

[a] In ratios listed, numerator is number of plants infected; denominator, number tested.

One other comparison which might be made is that concerned with virus source plants which were infected with a single virus compared with plants which were dually infected. Based on the total results in which comparable conditions within each of the four experiments were maintained, a total of 23 individual plants were tested as sources for each of the yellows and the yellow-net viruses, and a total of 22 individual plants were tested as sources of both viruses. From the 23 yellows infected plants, when the acquisition feedings were confined solely to these plants, 163 plants were infected out of 1071 tested (15.2%); while from the dually infected plants, 357 yellows infections occurred out of a possible 2146 tests (16.6%). From the 23 yellow-net sources, under similar conditions, 197 infections were obtained out of a possible 1052 plants tested (18.7%); while from the dually infected plants, 239 infections were obtained out of a possible 2146 (11.1%). A chi square comparison of the difference in yellow-net transmission yielded a value of $\chi^2=3.12$, d.f. 1, $p=>0.05$; but with the amount of within treatment heterogeneity that was evident, it would be difficult to attach any particular significance to this comparison. It probably would not be too far from actuality to assume that as a result of these tests, plants, whether single or doubly infected with the yellow and/or yellow-net viruses would serve as comparable virus source plants for green peach aphids, *i.e.*, differences in plants to serve as good or poor virus source plants, are not correlated with the presence of two viruses within the same host plant, in the case of yellows and yellow-net viruses in sugar beets, when using green peach aphids as the vector.

Of more general interest is the actual performance of the aphids during each 8-hour period spent on a new healthy test beet seedling. Presentation of the total data obtained would be cumbersome, but possibly it can be meaningfully summarized by the use of a table and a graph. Table 7 gives the results of the four experiments. In all cases the per cent transmission has been based on the expectancy of transmission by single insects, using the relationship $p=1-q^x$, where $p=$the probability of obtaining an infection, $q=$the probability of not obtaining an infection, and $x=$the number of insects. In presenting the graph (fig. 1), the actual points have not been

Table 7.—Per cent transmission in trials to determine the period of retention of the beet yellows and the beet yellow-net viruses by green peach aphids.[a]

Experiment Number	Virus Transmitted	Test Plant Number						
		1	2	3	4	5	6	7
1	Yellows	6.0[b]	3.5	3.0	1.5	0.5	0.0	0.0
	Yellow-net	2.5	4.5	5.0	4.5	3.0	7.0	6.0
2	Yellows	25.0	16.0	7.5	3.0	3.0	0.5	0.5
	Yellow-net	4.5	7.0	9.5	9.5	18.0	21.0	15.0
3	Yellows	26.5	23.5	13.5	8.0	6.0	3.0	2.0
	Yellow-net	4.5	2.5	5.0	6.0	7.0	11.8	9.5
4	Yellows	18.0	10.2	5.4	2.2	2.5	0.92	0.71
	Yellow-net	0.2	0.6	0.41	0.43	0.9	1.4	0.49

[a] The insects were fed on virus source plants for a total of 24 hours before being serially transferred at 8-hour intervals to a total of seven healthy test plants.

[b] The numbers in the columns are the per cent transmission by single insects. In experiments 1, 2, and 3, 10, 5, and 5, insects per plant were used, respectively. The expectance of transmission by single insects was calculated using the relationship $p=1-q^x$, where $p=$probability of transmission, $q=1-p$, and x is the number of insects. In experiment 4, single insects were actually used.

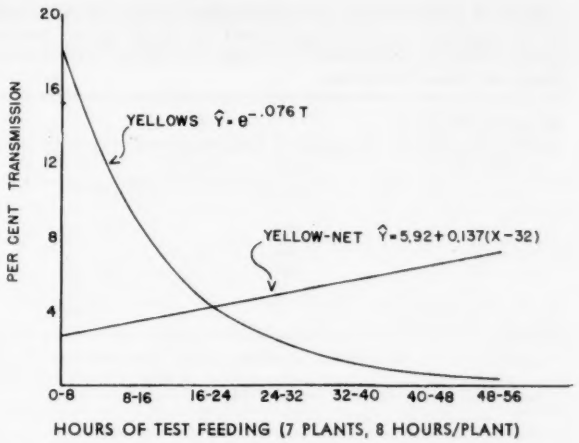

FIG. 1.—Theoretical curves illustrating the retention of the beet yellows and the beet yellow-net viruses by feeding infective green peach aphids.

plotted, but the graph is believed to represent one of the more likely expressions of the manner in which the yellows and the yellow-net viruses are transmitted to a series of healthy test plants by infective green peach aphids which are allowed 8 hours of test feeding on each plant. The curves were fitted using the least squares method. The equation for the transmission of yellows virus follows the expression $\hat{Y}=e^{-\alpha t}$, with alpha being a constant, and t being time; while in the case of the yellow-net virus, linearity was assumed, the regression line calculated using the equation $Y=a+bX$.

The probability of transmission of the yellows virus decreased in the manner of a half-life decay curve, the rate of decrease being constant and dependent upon the amount of virus present at the beginning of each feeding period. It is this relationship which provides the answer to the question as to why in the preliminary experiments on retention the insects with the yellow-net virus appeared to retain the yellows virus for a longer period of time than normal. Apparently it was because in that particular series the population of aphids contained more infective individuals in it, and the level of virus charge per individual was high in comparison to the other series.

Of interest also is whether one insect can carry and transmit both the yellows and the yellow-net virus. In the experiment using single insects, only 10 insects which had the opportunity to acquire both viruses during the acquisition feeding, out of 400 which were tested, transmitted the yellow-net virus. However, 4 of these 10 transmitted both the yellows and the yellow-net viruses, but in no case did the insects inoculate a plant simultaneously with both viruses. Therefore, while it was demonstrated that a single insect can carry both viruses at one time, it still is unknown whether a single insect can inoculate a plant simultaneously with the two viruses.

Another point which became evident during examination of the data was that single insects varied considerably in their charge of virus. If there was a constant probability of causing infection among the individual insects, the number of plants inoculated per individual should be predictable by the binominal theorem; or, if the probability of inoculation was low, then the Poisson distribu-

Table 8.—Fitted Poisson distribution for the distribution of the number of plants inoculated by single green peach aphids[a] serially transferred to a total of seven healthy test plants at 8-hour intervals.

Possible Number of Plants Which Could Be Inoculated	Number of Insects Inoculating Plants	
	Actual	Expected[b]
0	162	136.51
1	35	71.21
2	23	18.58
3	5	3.22
4 or more	5	0.48

[a] Aphids were allowed a 24-hour acquisition feeding period on virus source plants infected with either beet yellows virus alone, or dually infected with both the beet yellows and beet yellow-net viruses before being transferred.

[b] The expected numbers were calculated using data obtained from 230 insects fed on 1610 plants. 68 insects proved infective, and these inoculated 120 plants.

tion should obtain. In table 8, the results are given of an attempt to determine whether the level of charge per infective individual was a constant. Testing the goodness of fit by chi square analysis yielded a value of $\chi^2 = 34.95$, d.f. 2, $p = <0.01$. For purposes of analysis, the category of 4 or more infections was combined into the category of 3 infections in order to avoid biasing the chi square value with an extremely small denominator (expected number of infections). It is evident from the results that the probability of causing an infection was not a constant. Apparently, the individual aphids varied greatly in their level of virus charge, or their ability to successfully inoculate plants. This is one difference between the beet yellows virus and some of the typically nonpersistent viruses in their vector-virus relationships, for it has been demonstrated in the case of Henbane mosaic and lettuce mosaic, that approximately equal charges of virus are obtained during a limited acquisition feeding, one designed to insure maximum infectivity of the aphids (Bradley 1952; Sylvester 1955).

Some mention should be made of the gradual increase in transmission expectancy of the yellow-net virus as the serial transfers progressed. This increase might indicate a latent period, or perhaps an adjustment period required by the aphid to resume normal feeding (Kirkpatrick & Ross 1952). In any case, it seems to be associated with the test plants. Phenomena of this type have been found by Chaudhuri (1950) with pea-enation mosaic and *Macrosiphium pisi* (Kalt.), by Kirkpatrick & Ross (1952) in potato leaf-roll studies, and by Prentice (1952) in connection with strawberry crinkle virus. It is this type of phenomenon which lead Bawden (1950) to suggest that latent periods were associated with the test plants. A need for clarification of this point by additional research is indicated.

Post-acquisition Fasting Retention.—The next consideration is whether the retention of beet yellows virus by the green peach aphid during fasting periods is similar to that when the insects are feeding. Watson (1946) reported that the rate of decrease of infectivity of infective green peach aphids during a post-acquisition fasting period was in excess of that during a comparable feeding period. This was in contradistinction to the phenomenon found in connection with beet mosaic virus transmission by the same species (Watson 1946), and other nonpersistent viruses upon which such data have been reported (Sylvester 1954). Thus, it seemed that this conclusion was worth rechecking, since it should be considered in evaluating the factors involved in the vector-virus relationships among the persistent and the nonpersistent aphid-borne viruses.

The effect of post-acquisition fasting was tested using the leaf cage technique. A total of 15 replications, 10 plants per treatment, 4 aphids (apterae and late instar nymphs reared on *Brassica juncea*) per plant, were done. The post-acquisition intervals tested were: 1, 2, 3, 4, 5, 6, 8, and 24 hours. Two virus source plants were used. The temperature recordings at hourly intervals during the test intervals indicated that the laboratory temperature was $22.4 \pm 1.1°$ C. The acquisition feeding was 15.77 ± 0.99 hours, and the following test feeding periods obtained: test feeding period for the insects fasted 1 hour after the acquisition feeding, 23.91 ± 0.98 hours; those fasted 2 hours, 22.91 ± 0.98 hours; 3 hours, 21.91 ± 0.98; 4, 5, 6, and 8 hours, 1 hour less each (except 8, which was 2 hours less than 6) with the same value for the standard deviation, and those fasted for 24 hours after the acquisition feeding, the test feeding period was 24.78 ± 1.13 hours. The reason for the hour decrease in the 1 through 6 intervals and the 2-hour decrease in the 8-hour interval was that all the insects were removed at the same time the day after the test feeding was begun. However, since the inoculation threshold period data indicated that a maximum transmission level occurred within 8 hours, it was felt that it was unlikely that the variation in the test feeding periods influenced the results.

A record was kept of the number of aphids which were dead in each cage of four at the end of the test feeding period. If the rate of death varied according to the fasting treatment, the factor should be considered in interpretation. For simplicity, linearity was assumed in the rate of death, and if near maximum transmission occurred during the first 6 hours of test feeding, and the number of aphids alive after 3 hours of test feeding calculated, then a mean number of aphids alive per group of 4 during the first 6 hours of test feeding can be derived. With these assumptions, it was calculated that the mean number of aphids alive during the critical first 6 hours of test feeding for the various treatments was: 1 hour post-acquisition fasting, 3.92; 2 hours post-fasting, 3.90; 3 hours, 3.87; 4 hours, 3.82; 5 hours, 3.87; 6 hours, 3.86; 8 hours, 3.82; 24 hours, 3.83. There was little evidence that the length of the post-acquisition fasting period caused a serious variation in the ability of the individuals actually used in the test to survive.

Based upon the number of plants infected, a chi square value of 57.02, d.f. 14, $p = <0.01$, indicated a lack of homogeneity between replications. The results of the experiment are given in table 9, and a graphic interpretation of the results are given in fig. 2. It is evident that there was a limit to the infectivity which could be expressed by infective individuals under the conditions of the experiment. The amount of transmission obtained in the intervals 1 through 6 was examined for homogeneity by chi square, and no evidence was obtained to reject the hypothesis that the samples were drawn from the same population ($\chi^2 = 0.07$, d.f. f, $p = >0.99$). Assuming linearity, and fitting a regression line to the results for the first

Table 9.—Results[a] of trials to determine the retention of the beet yellows virus by infective green peach aphids during periods of post-acquisition fasting.

Hours of Post-Acquisition Fasting								
1	2	3	4	5	6	8	24	Total
23/150	35/150	28/150	27/150	28/150	33/150	12/150	1/115	181/1165

[a] In ratios listed, numerator is number of plants infected; denominator, number tested.

6 intervals, the value $\hat{Y}=1.93+0.229\ (X-3.5)$ was obtained. For purposes of the graph (fig. 2), an average per cent transmission of 20 with slope 0, for the intervals 1 through 6, was assumed. The final curve $Y=e^{-\alpha t}$, was then fitted using 3 points, the values obtained at 6, 8, and 24 hours, assuming the maximum expression of transmission to be unity.

When this was done, it was possible to arrive at a figure of approximately 2.55 hours as an estimate of the half-life of the yellows virus in fasting green peach aphids. This was a more rapid rate of disappearance of infectivity than was found under conditions of feeding. In feeding insects the half-life estimation approximated 8 hours. It is regrettable that more predictability of the results was not available when the experiment was designed, for this would have allowed the establishment of more points in the critical area, *viz.*, the hours between 8 and 24 hours. However, it is believed that the data are strong enough to confirm the conclusion reached by Watson (1946); namely, that the loss of infectivity of beet yellows virus in fasting green peach aphids exceeds that in feeding insects. Whether approximately 2½ hours is a proper estimate of the rate of loss in fasting insects will have to be judged by additional data.

INFLUENCE OF THE NUMBER OF APHIDS ON TRANSMISSION.—Throughout the work, one of the assumptions which was made concerning the transmission of the beet yellow virus by green peach aphids, was that the infections by individuals within a group were separate and independent events, and that in any one population sample there was a constant probability of virus transmission. The standard method of testing this assumption is to compare the transmission of virus by groups of insects which vary in number, and then determining whether the results can be fitted to the binominal expectancies using the relationship $p=1-q^x$.

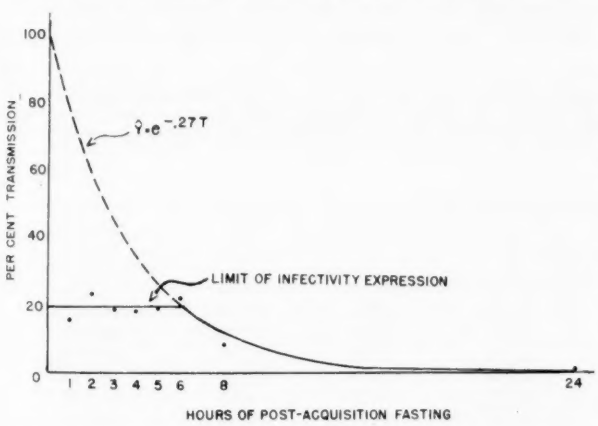

FIG. 2.—Theoretical curve illustrating the loss of infectivity with the beet yellows virus by fasting infective green peach aphids.

In order to test the effect of groups of green peach aphids on the transmission of the beet yellows virus to healthy beet seedlings, two experiments were done. In the first experiment, using 6 replications, 20 plants per treatment, the test plants were fed on by the following groups of infective green peach aphids: 1, 2, 4, 6, 8 and 10. The virus source was a field collected beet upon which the vectors were reared. Apterae and late instar nymphs were used. The minimum acquisition feeding in replications 1, 2, and 3, was 3 days, while in replications 4, 5, and 6, it was 8 days. The test feeding period was 23.93 ±0.08 hours. The insects were collected by aspiration directly into glass vials (32×9 mm.) which were then inverted over the test plants. In the second experiment, consisting of 8 replications, 10 plants per treatment (with the exception that limitations in the numbers of available aphids limited the number of plants per treatment to 7 in replication 4, and 8 in replication 7), was done in a similar manner. In this experiment, the aphids were reared as non-infective insects on either sugar beet or mustard plants, and were fed on virus source plants (a total of 10 different virus source plants being used), for an acquisition period of 20.79±2.89 hours, before being collected and given access to the test plants for a period of 24.24 ±0.4 hours.

A record was kept of the number of insects on the test plants at the end of the test feeding, but examination of these data indicated that there tended to be a constant probability of aphids not present on the plants at the end of the test feeding period, and it was judged that adjustment of the data on this basis would only change the quantitative aspects of the results, not the qualitative.

The results of the two experiments are summarized in table 10. It is evident that the probability of obtaining an infection with a group of aphids is in agreement with the assumptions of constant probability for infection within the population and that each infection is a separate and independent event. A chi square analysis of the number of plants infected per group and the number of plants

Table 10.—The influence of the number of green peach aphids per test plant on the amount of transmission of the beet yellows virus.[a]

Experiment[b]	Number of Aphids per Test Plant						Total
	1	2	4	6	8	10	
1	0/111	4/114	8/113	7/112	10/113	15/114	44/677
2	5/75	3/73	7/72	13/75	16/73	17/73	61/441

[a] In ratios listed, numerator is number of plants infected; denominator, number tested.

[b] Experiment 1 consisted of 6 replications, 20 plants per treatment; while experiment 2 consisted of 8 replications, 10 plants per treatment (except replications 4 and 7, which had 7 and 8 plants per treatment, respectively).

expected to be infected per group, using the binominal theorem as the basis for calculating the expected, lead to a chi square value of 0.81, with d.f. $(n-2)4$, $p = >0.90$) No statistical support could be gained for the rejection of the hypothesis of independence and constant probability of infection.

One of the reasons for emphasizing this point is that results obtained by Kirkpatrick & Ross (1952) using the green peach aphid and the persistent virus, potato leafroll, indicated that there was an unfavorable interaction between numbers of aphids and virus transmission, which lead to the suggestion that possibly the feeding of aphids on plants decreased the susceptibility to virus infection (Simpson & Shands 1949, Kirkpatrick & Ross 1952). In the case of Kirkpatrick & Ross, the test feeding periods were prolonged, a matter of days. Work with a non-persistent virus (lettuce mosaic) and the green peach aphid (Sylvester 1955) failed to support this hypothesis, but it was felt that the point was worth reëvaluating in connection with the work on the beet yellows virus, especially when a number of the experiments involved the use of groups of insects.

In spite of the fact that the first two experiments failed to support the hypothesis of aphid numbers reducing plant susceptibility, additional tests were done, experiments which involved small populations of non-infective as well as infective aphids.

In order to test the influence of combinations of infective and non-infective green peach aphids on the transmission of the beet yellows virus, the following experiment was repeated 31 times. Green peach aphids (apterae and late instar nymphs), reared on a mustard plant, were collected by aspiration in lots of 10 to 20 in 60 leaf cages. Thirty of the leaf cages were placed on a virus source plant, and the remaining 30 cages were placed on leaves of a healthy beet plant. The selection of the individual cages was at random. The acquisition feeding, with a value of 23.96 ± 0.1 hours in length, was done in a soil house where the temperature was near that of out-of-doors, and the light intensity was somewhat reduced. This was done in an attempt to reduce mortality. The following day, the cages were removed and the insects combined at random into leaf cages in accordance to a factorial design using all combinations of 0, 1, 2, 4, 6, 8, and 10 infective and non-infective insects per cage. Each cage was placed in a labeled envelope and placed in a box, and then the cages were put on the test plants at random. After a test feeding of approximately 24 hours (21.14 ± 1.01 hours), the cages were removed at random, and the plants were sprayed with nicotine. The insects which were alive in the cages were counted.

The results of the trials are given in table 11. The first examination of the data was as to whether or not increasing the number of non-infective aphids had a tendency to reduce virus transmission. Although the chi square value of 11.9, d.f. 6, $p = >0.05$, indicated a tendency for heterogeneity to exist among the series, there was no tendency for the lowest values to be positively correlated with the increasing number of aphids per feeding area. An attempt also was made to determine if mortality was associated with the increasing numbers of aphids, but a chi square test, ($\chi^2 = 2.15$, d.f. 5, $p > 0.70$) indicated that the variation encountered could be expected from chance. Non-infective aphids and infective aphids alone were found to have approximately the same mortality. With these judgments as a basis, it was assumed that non-infective aphids had no influence on the ability of infective aphids to inoculated beet plants with the beet yellows virus during a 24-hour test feeding period, and that mortality, when it occurred, was randomly distributed throughout the experiment. Thus, mortality would cause random quantitative variations in the data. If it is assumed that the crucial period of time for the inoculation of the plants with beet yellows virus is during the first 6 hours of test feeding, then the mortality factor is still further reduced in consequence.

The next level of evaluation was that concerned with the actual transmission by numbers of infective insects. There were various ways to estimate a $\hat{p}$ value for the expectancy of transmission by single insects. One method was to use the value actually obtained in the series of tests involving only one insect, of $\hat{p} = 0.0837$. This gave the highest value for p. Another way was to calculate the expected $\hat{p}$ based on the total number of transmissions, 275 infections out of 1287 tests, with a mean of 5.167 aphids per test, which resulted in a value $\hat{p} = 0.0454$. A third method was to reduce the results of every test to the expectance for a single insect, and calculate a mean p value and its standard deviation. When this was done, the value for $\hat{p} = 0.0559 \pm 0.027$ was obtained, which gave

Table 11.—Results[a] of trials with green peach aphids to determine whether the presence of non-infective individuals feeding in the same locale of infective individuals influences the probability of obtaining infection with the beet yellows virus.

Number of Non-Infective Aphids per Lot	Number of Infective Aphids per Lot							Total
	0	1	2	4	6	8	10	
0	0/31	3/29	5/30	3/30	6/30	13/30	10/30	40/210
1	0/31	3/31	4/31	6/31	7/31	11/31	11/30	42/216
2	0/30	4/31	4/30	5/31	7/31	12/31	8/31	40/215
4	0/31	1/31	2/31	5/31	8/31	6/30	12/31	34/216
6	0/31	3/31	2/31	6/30	4/30	16/30	12/30	43/213
8	0/31	2/31	7/31	8/31	6/31	8/31	4/31	35/217
10	0/31	2/31	4/30	5/31	9/31	8/31	13/31	41/216
Total	0/216	18/215	28/214	38/215	47/215	74/214	70/214	275/1503

[a] In ratios listed, numerator is number of plants infected; denominator, number tested. Variation in denominator due to death of test plants.

a one standard deviation range of $p=0.0289$ to 0.0829.

Regardless of the p value selected for use, it was inescapable to note that there was a tendency for the data not to fit the expectancy as predicted using the relationship $p=1-q^x$, for in this series of tests, single insects had the highest probability of transmission, while the groups of 10 infective insects had the lowest probability of transmission per individual. This could well be used to support the hypothesis that the probability of transmission by groups is not a constant, and that the reason is that the feeding of numbers of aphids had a tendency to decrease the susceptibility of the host plant (Simpson & Shands 1949, Kirkpatrick & Ross 1952). However, in the case of the present experiments with beet yellows and the green peach aphid, perhaps the decrease in plant susceptibility could only be shown to be probable in the case of infective insects, and was independent of non-infective groups. However, in the opinion of the author, the within treatment variability, due to interaction effects of the various infective-non-infective combinations, was too great to permit this conclusion, and it would take far more rigorous testing than was done in this experiment to either support or deny the hypothesis. This is especially true when considered against the background of the other experimental work in which the transmission of beet yellows by groups of green peach aphids was tested without the complication of the addition of non-infective aphids to the lots. In this case, as shown previously, the groups of aphids responded as predicted by the hypothesis that infections are separate and independent events and that there is a constant probability of inoculation.

DISCUSSION.—During the experimental work with beet yellows virus and the green peach aphid, it has become increasingly apparent that the vector-virus relationships are not strictly of the persistent type (Watson & Roberts 1939). The rate of infectivity loss is too rapid (Smith 1931, Osborn 1935, Smith & Lea 1946, Sylvester 1949, Chaudhuri 1950, Anderson 1951, MacCarthy 1954, Simons 1954), and post-acquisition fasting is too detrimental to infectivity (Sylvester 1950, Anderson 1951, Simons 1954). On the other hand, the vector-virus relationships are not those conceived as nonpersistent (Watson & Roberts 1939, Sylvester 1954). While it is true, the type of curves which might be used to estimate the manner in which acquisition, inoculation, and retention occur, differ mainly in quantitative rather than qualitative aspects (Smith & Lea 1946), the fact remains that in the case of beet yellows preliminary fasting fails to increase efficiency (Watson 1946), and post-acquisition fasting shortens retention rather than prolonging it (Watson 1946).

Because of these differences, it has been proposed that beet yellows, in its vector-virus relationships, is representative of a group of *semi-persistent* viruses (Sylvester 1956), implying that this group is, in aphid transmission characteristics, in between the nonpersistent and the persistent groups. But it should be recognized that this may be but a convenient way of classifying fragments of a continuum representing a portion of the phylogeny of vector-virus relationships. To date, comparatively little is known about this group of semi-persistent viruses, but other possible examples are cauliflower mosaic (Hamlyn 1955), dandelion yellows mosaic (Kassanis 1947), and some of the strawberry viruses (Prentice & Harris 1946). Much more data are needed before the limitations and variability of the characteristics of the vector-virus relationships within the semi-persistent group can be defined. In fact, additional data are needed on all the groups of aphid-borne viruses, as well as the other arthropod vector groups, if understanding is to be achieved.

SUMMARY AND CONCLUSIONS.—During experiments on the vector-virus relationships between beet yellows virus and the green peach aphid, *Myzus persicae* (Sulz.), in which some work on the transmission of the beet yellow-net virus also was done, it was found that the acquisition and inoculation threshold periods for yellows virus probably should be considered in terms of minutes, with maximum levels of efficiency being determined in hours. The minimum period for virus acquisition was 1 hour, with maximum efficiency of virus acquisition occurring after approximately 12 hours of feeding. The minimum interval determined for the inoculation threshold period was 1 hour (the minimum interval tested), with maximum inoculation efficiency being attained in about 6 hours.

In retention experiments, the maximum period of time that yellows virus was retained was 3 days; but as a result of experiments involving both the yellows and the yellow-net viruses in serial transmission sequences, it was found that yellows virus had a half-life within feeding aphids of about 8 hours. During the 8-hour serial transfers, transmission of beet yellow-net virus gradually increased, indicating that either a latent period existed, or that the ability to transmit the yellow-net virus was affected by some conditioning factor associated with the test plants and/or the test feeding.

Yellows virus was retained by fasting infective aphids for the maximum interval tested (24 hours), but the data suggested that yellows virus had a half-life of approximately 2.5 hours in fasting insects. Thus, the rate of loss of infectivity was more rapid in fasting than in feeding infective aphids.

In spite of an undesirable low level of virus contamination of some of the non-infective stocks of insects, the factorial experiments on retention and their subsequent analysis lent some support to the following conclusions. Single insects were capable of carrying the yellows and the yellow-net virus at the same time. It was not established that single insects could inoculate an individual beet plant with both viruses during any one 8-hour test feeding period. However, on numerous other occasions, using lots of insects, individual plants were infected with both the yellows and the yellow-net viruses. Failure to obtain dual inoculation with single insects apparently was due to the low probability of transmitting the yellow-net virus in these trials, rather than being due to any intrinsic mechanism within the insect which would prevent both viruses being ejected simultaneously.

With yellows virus, leaves without evident symptoms tended to be superior sources of virus when compared with leaves exhibiting symptoms. An opposite tendency was indicated in the case of the yellow-net virus.

When virus transmission was effected from plants infected with both the yellows and the yellow-net viruses. it was of little consequence to transmission whether or not the symptoms of either disease were present on the leaves used for the acquisition feeding. Individual dually

infected plants varied considerably in their capacity to serve as virus sources, but superiority of any one plant as a source of one of the viruses did not assure that it would be a superior source of the other.

There were no indications that the acquisition of either the yellows or the yellow-net virus before acquisition of its opposite influenced the ability of the insects to transmit. However, if the terminal acquisition feeding was on a plant infected with the yellow-net virus, there was a loss of yellows infectivity. Thus, the effect on vector efficiency of a 12- or 24-hour feeding period on a yellow-net source was comparable to a like period spent on a virus-free plant.

In experiments designed to determine the influence of numbers of infective aphids on the probability of transmission, it was found that when a design was limited to testing infective groups alone, the probability of transmission was constant, and each infection could be considered as a separate and independent event. When an additional variable of groups of non-infective aphids was introduced into the design, the probability of infection tended not to be constant, but changes in this probability could not be associated with non-infective members of the test groups. Possible interaction factors and small sample size and the leaf cage technique appeared to be responsible for the variability of the data.

Based upon comparative characteristics, it is proposed that the beet yellows virus is representative of a *semi-persistent* group of aphid-borne viruses, a group which appears to be distinct from, yet in some ways similar to, both the non-persistent and the persistent groups of aphid-borne plant viruses. The existence of such a group lends support to the concept of a vector-virus relationship continuum among the aphid-borne plant viruses.

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The Metabolism of P^{32}-Labeled Bayer L 13/59 in a Cow[1]

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The insecticide Bayer L 13/59, the chemical name of which is *O,O*-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate, has attracted considerable attention during the last two years. Bruce (1953), Gahan *et al.* (1954) and other workers have shown it to be very effective in baits against DDT-resistant house flies.

The authors were interested in Bayer L 13/59 (Dipterex) because of its low mammalian toxicity (DuBois & Cotter, 1955, Deichmann & Lampe 1955) and effectiveness against cattle grubs. Research by McGregor *et al.* (1954) demonstrated that the compound would kill *Hypoderma lineatum* DeVill. when administered orally to the host. Smith & Richards (1954) and Roth & Eddy (1954) also found it effective against encysted grubs (*lineatum*) when applied as a wash to the backs of cattle. However, this research further indicated that the material was lethal only to the grubs that had encysted in the backs of the animals. It failed to kill the smaller grubs present in the gullet or other parts of the body.

Recent work has demonstrated that in alkaline media L 13/59 undergoes dehydrochlorination and rearrangement to form the compound *O,O*-dimethyl *O*-2,2-dichlorovinyl phosphate known as DDVP, which has also been reported to be present in trace amounts in the technical material (Mattson *et al.* 1955, Barthel *et al.* 1955, Lorenz *et al.* 1955).

In order to gain a better understanding of the relationships that exist between the host, parasite, and toxicant, and to study the metabolic fate of the compound, P^{32}-labeled L 13/59 was administered orally to a grub-infested cow. At intervals following treatment grubs and exudate from the grub cysts were removed and samples of blood, urine, feces, and milk taken and assayed radiometrically and/or chromatographically.

MATERIALS AND METHODS.—*Experimental Animal.*—The animal used in this study was a lactating Hereford cow. At the time of administration of the labeled compound the cow weighed 920 kg. and had in its back 12 third-stage grubs of *H. bovis* (L.).

Radioactive Compound.—The P^{32}-labeled L 13/59 was synthesized by Acree *et al.* (1956). At the time of administration the specific activity was 4.99×10^9 cpm/gram, as calculated from two weighings. One gram of this material was added in solution to 4.5 grams of nonradioactive L 13/59 (m.p. 78°–80.5° C. uncorr.) to give a total of 5.5 grams of the diluted compound with an observable specific activity of 9.07×10^8 cpm/gram. The radiochemical purity of the undiluted labeled L 13/59, as determined by two chromatographic methods of analysis (xylene-Carbowax and benzene-triethylene glycol systems), was 90.1% (mean of 11 chromatograms). The major impurity (9.8%) remained at the point of application by both methods, and less than 0.1% of the radioactivity ran with R_f of DDVP.

Administration of Radioactive Compound.—The labeled L 13/59 was administered orally at the rate of 25 mg./kg. in 100 ml. of distilled water. Following administration, the flask and the syringe were rinsed with five 75-ml. aliquots of water, which were also administered to the animal.

Sample Collection.—The animal was held in a chute, and food and water were provided throughout the experimental period. Urine and feces were collected manually and considerable care was taken to avoid cross contamination. Near-quantitative collections of all urine and feces excreted were made for the first 12 hours, but subsequent samples were taken only at the intervals indicated.

Samples containing 8 to 10 ml. of venous blood were collected with a 10-ml. glass syringe. Sodium citrate was used as the anticoagulant.

The animal was milked manually after the udder had been carefully washed and checked with a survey meter. An attempt was made to remove all the available milk at each sampling.

Following collection and radiometric analyses, formalin was added to the blood, milk, and urine as a preservative, and the samples were held under refrigeration until extractions and/or chromatographic analyses could be performed.

The grubs were removed by applying pressure with the fingers around the base of the cysts. Any exudate present in the cyst was removed with a dropper and applied to a tared planchet and the net weight determined.

Radiometric Measurements and Sample Preparation.—The radiometric analyses were made with a conventional scaler and a windowless, gas-flow proportional counter. All samples were prepared in triplicate, and the mean corrected counting rate was used in computing the radioactivity. The samples were counted for a sufficient time to attain a minimum standard errror of $\pm 5\%$ (Calvin *et al.* 1949). The counting rates were corrected for decay and expressed as microgram- or milligram-equivalents of L 13/59.

Samples of blood, urine, and milk were mounted by plating aliquots on aluminum planchets. To insure even distribution, discs of tissue paper 2 cm. in diameter were attached to the bottom of the planchets. Care was taken to keep the sample thickness below the level of appreciable self-absorption. To prevent losses during drying and variations in the background rate due to contamination of the windowless counter, 2 to 3 mg. of Carbowax 400 (polyethylene glycol) was added to the samples.

The grub exudates were assayed by use of weighed quantities. The feces was treated in the following manner: The entire sample was thoroughly mixed for 10 minutes with an electric stirrer, and then 2-gram portions were weighed to the nearest 0.01 gram and wet-digested with nitric acid. The digests were adjusted to known volumes and measured aliquots plated and assayed.

Because of the small amount of radioactivity present

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and the difficulties encountered in extractions, the grubs were wet-digested. When control grubs and feces to which a known amount of P^{32}-labeled L 13/59 had been added were digested in the same manner, recoveries ranging between 88 and 95% were obtained.

Analyses.—Both paper chromatographic and biological methods of analysis were employed. The following chromatographic systems were used:

(1) Xylene-Carbowax 400.—One-inch strips of Whatman No. 1 paper were uniformly coated with Carbowax 400 by passing them through a 5% (v/v) acetone solution. After thorough drying (5 to 10 minutes) the solutions to be analyzed were applied to the strips and the chromatograms were developed by the ascending technique, with xylene (CP) saturated with Carbowax 400 as the mobile phase.

(2) Benzene-triethylene glycol.—Strips of Whatman No. 1 paper were coated with the stationary phase by using a 2.5% (v/v) acetone solution of triethylene glycol. The mobile phase consisted of benzene (CP) saturated with triethylene glycol. Analysis was performed as described for the xylene-Carbowax system.

(3) Ethanol-hydrochloric acid.—The developing solvent consisted of 7.5 parts of 95% ethanol to 2.5 parts of 0.2% of concentrated hydrochloric acid (v/v). The solutions to be analyzed were applied to 1-inch strips of Whatman No. 1 paper. The strips were allowed to equilibrate in a chamber containing the solvent for about 1 hour. The sides of the chamber were lined with filter paper immersed in the solvent to insure saturation of the atmosphere. After equilibration the strips were lowered into the solvent and the chromatogram was developed by the ascending method.

R_f values for L 13/59 and related compounds by all three methods are reported in table 1. They were determined colorimetrically and/or radiometrically. L 13/59 and DDVP were detected by spraying the developed chromatograms with an alkaline permanganate solution (potassium carbonate 3% potassium permanganate 0.4%); the presence of the compounds was detected by a brown spot (MnO_2) on a pink background. This method is nonspecific and relatively insensitive, and was used only in the development of methods and in establishing R_f values. Dimethyl hydrogen phosphate, dimethyl hydrogen phosphite, orthophosphoric acid, and the mammalian metabolite(s) were detected by the colorimetric technique of Hanes & Isherwood (1949). Radiometric analyses were performed by cutting the developed chromatograms into 1-cm. strips and assaying them in a windowless proportional counter.

A 20 to 30 cm. development was employed for all systems. The xylene-Carbowax system separates L 13/59 and DDVP. The latter compound runs very near the front by the benzene-triethylene glycol method, but this technique gives a better separation of L 13/59 and its metabolic products. The ethanol-hydrochloric acid system was used to separate the two major metabolites, which behaved as a single compound when analyzed by the other methods. The R_f's reported for this system were determined in the presence of control urine (10 to 15 microliter aliquots) to take into account R_f shifts due to interfering substances.

Table 1.—R_f values for Bayer L 13/59 and related compounds at 23–25° C.

Compound	Xylene-Carbowax 400	Benzene Triethylene Glycol	Ethanol-HCl
L 13/59	0.25	0.37	0.79
DDVP	.87	.98	.81
Dimethyl hydrogen phosphate	—	—	.47
Dimethyl hydrogen phosphite	—	—	.47
Orthophosphoric acid	—	—	.05
L 13/59 metabolites (in urine)	.02	.01	.47
			.23

The blood and milk were prepared for chromatographic analysis by adding an excess of acetone (5 volumes) to precipitate the proteins. Following filtration, the acetone was removed by passing a gentle air stream over the filtrates. The aqueous fraction of the blood was analyzed directly. The milk filtrates were extracted five times with equal volumes of chloroform, and pooled extracts were analyzed radiometrically and chromatographically. Because the radioactive material in the urine could not be extracted with any of a large number of organic solvents tested, aliquots of whole urine were analyzed.

The bioassay method employed to assess the toxicity of the radioactive compounds present in the milk was essentially the feeding technique of Sun & Sun (1953) with the following modifications: 3- to 4-day-old female house flies of a normal strain were employed, 50 for each determination, and the sample to be evaluated was placed in a petri dish containing cotton.

RESULTS.—*Blood.*—Thirty minutes after administration the concentration of radioactivity in the blood was 8.8 μg-equivalents/ml. (fig. 1). The peak of radioactivity was attained between the first and third hours after treatment, with a recorded maximum of 15.1 μg-equivalents/ml. at 2 hours. Following the peak the radioactivity decreased at a constant rate until after 24 hours it was only 0.55 μg-equivalent/ml.

Repeated extraction with chloroform (5 equal volumes) of the acidified protein-free filtrates prepared from the blood samples removed only a small percentage of the

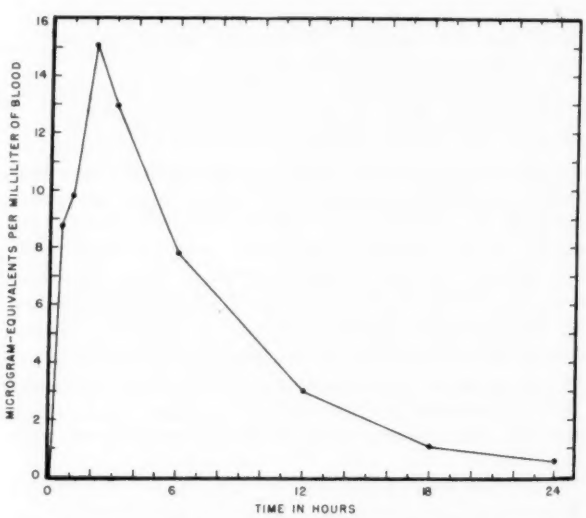

FIG. 1.—Radioactivity in the blood of a cow following oral administration of P^{32}-Bayer L 13/59.

Table 2.—Bayer L 13/59 in bovine blood as determined by paper chromatographic analyses of protein-free filtrates prepared by the benzene-triethylene glycol system.

INTERVAL (HOURS)	μG.-EQUIVALENTS/ML.	PER CENT	μG./ML.
0.5	8.8	2.5	0.2
1	9.8	4.9	.5
2	15.1	7.5	1.1
3	13.0	6.8	.9

radioactive compounds. These poor extractions indicated that very little of the radioactivity in the blood was unchanged L 13/59. This was further substantiated by paper chromatographic analyses of the ½ through 3-hour blood samples by the benzene-triethylene glycol system. By this method only a small percentage of the radioactivity present in the blood samples ran with the R_f of L 13/59 (table 2). A maximum concentration of 1.1 μg. of L 13/59 per ml. of blood was found in the 2-hour sample. In all the samples the remainder of the radioactivity remained near the point of application (table 1). No DDVP was detected in any of the blood samples.

Grub Exudate.—Sufficient exudate for sample preparation was present only in the cysts of grubs removed 6, 12, and 192 hours after treatment. The results of radiometric assay of these samples are presented in table 3.

Table 3.—Radioactivity in exudates removed at intervals from grub cysts after administration of Bayer L 13/59.

INTERVAL (HOURS)	WEIGHT OF SAMPLE (MILLIGRAMS)	μG.-EQUIVALENTS/GRAM
6	95.9	7.7
12	27.8	6.3
192	21.0	0.9

They are presented as μg-equivalents per gram of exudate to allow for comparison with the blood-activity levels. From these limited data there appears to have been a greater retention of the radioactivity in the exudate than in the blood. Because of the small samples and the large quantity of interfering substances present, no chromatographic analyses were made of the exudate samples.

Grubs.—No radioactivity was detected in grubs removed before 3 hours (table 4), and the highest concentrations were present in those collected between 6 and 24 hours after treatment. Trace amounts of radioactivity were still present in the grub removed after 192 hours. Since the grubs were wet-digested, no chromatographic analyses could be performed.

Table 4.—Radioactivity in cattle grubs[a] removed at intervals after administration of labeled Bayer L 13/59.

INTERVAL (HOURS)	WET WEIGHT OF GRUBS (GRAMS)	μG.-EQUIVALENTS/GRAM
0.5	0.895	—
1	1.478	—
3	1.305	0.18
6	1.226	.98
12	1.117	1.03
18	.942	1.02
24	1.088	.86
48	.832	.38
120	1.452	.20
192	1.230	.13

[a] Samples prepared by wet digestion.

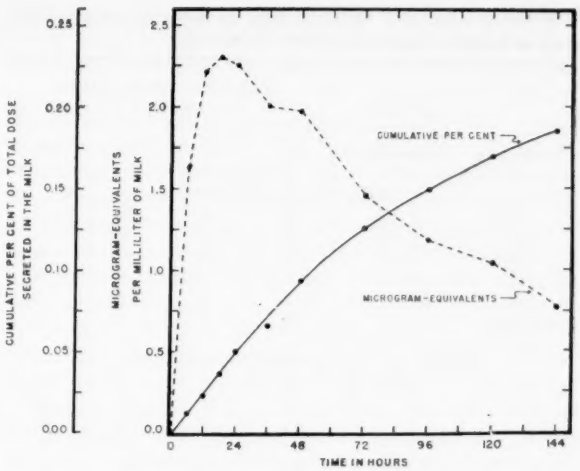

FIG. 2.—Radioactivity in the milk of a cow following oral administration of P^{32}-Bayer L 13/59.

Milk.—Relatively low levels of radioactivity were found in the milk (fig. 2). The maximum concentrations were detected in the 6–48 hour samples, with a peak of 2.3 μg.-equivalents/ml. at 18 hours; there was a constant decrease to 0.77 μg.-equivalent/ml. at the end of 144 hours, at which time less than 0.2% of the administered dose, as determined radiometrically, had been secreted in the milk.

When a 6–48 hour composite milk sample was extracted with five equal volumes of chloroform, as previously described, less than 10% of the radioactivity partitioned into the chloroform. When control milk samples to which an equivalent amount of L 13/59 had been added were extracted in the same manner, about 90% of the radioactivity partitioned into the chloroform. Paper chromatographic analyses of the chloroform extracts of the composite milk sample by the xylene-Carbowax and benzene-triethylene glycol systems demonstrated that only trace amounts of the extracted radioactivity ran with the R_f of L 13/59. None of the radioactivity in the chloroform extracts ran with the R_f of DDVP.

To determine if any of the radioactive phosphorus present in the milk was in the inorganic form, the casein was precipitated by adjusting a portion of the composite milk sample to a pH of 4.8. The sample was centrifuged and the supernatant decanted and treated with a "magnesia mixture" to precipitate the inorganic phosphorus present. The precipitate was thoroughly washed and then redissolved in a known volume of acid solution. By radiometric analysis it was found that 23.5% of the radioactivity was in the precipitate.

These findings are confirmed by the results of bioassays of the milk samples (table 5). These data indicate that the metabolic products of L 13/59 present in the milk were nontoxic to house flies at the concentrations present when assayed by a feeding technique.

Urine.—L 13/59 and/or its metabolites are very

Table 5.—Bayer L 13/59 in milk as determined by bioassays of female house flies 3 to 4 days old.

Interval	L 13/59 μg.Equivalent/Ml.	Per Cent Mortality After (Hours) 48	72	96	120
	Milk from treated cow				
6	1.63	0	0	2	2
12	2.21	0	0	2	2
18	2.30	0	0	0	0
24	2.25	0	0	0	0
36	2.01	2	2	2	2
Control		0	0	0	2
	P^{32}-Bayer L 13/59 added to control milk				
	1 μg./ml.	0	2	4	12
	2	2	24	36	42
	3	24	58	76	90
	4	56	84	94	100
	5	84	98	100	—

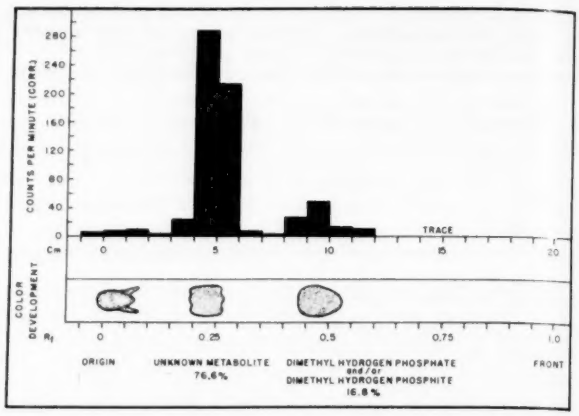

FIG. 4.—Paper chromatogram of composite urine sample by ethanol-HCl system, color development by the Hanes-Isherwood method. Percentages are the means of 4 runs.

rapidly excreted via the urine, as evidenced by figure 3. The first urine, excreted $1\frac{1}{4}$ hours after treatment, contained 0.54 mg.-equivalent/ml. The peak was attained at $2\frac{1}{2}$ hours, with a concentration of 1.4 mg.-equivalents/ml. At the end of $5\frac{1}{2}$ hours the concentration was still 1.38 mg.-equivalents/ml. and approximately 44% of the radioactivity of the administered dose had been accounted for in the urine. There followed a gradual decrease until, at the end of 24 hours the concentration was 0.026 mg.-equivalent/ml. During the first 12 hours nearly 66% of the radioactivity of the administered dose was accounted for in the urine.

Chromatographic analyses of the urine by both the benzene-triethylene glycol and the xylene-Carbowax systems demonstrated that less than 0.5% of the radioactivity in any of the samples collected during the first 12 hours was unchanged L 13/59. When the composite urine sample was analyzed chromatographically, only 0.26% of the radioactivity excreted during this period, or 0.17% of the administered dose, was found to be unchanged L 13/59. The rest of the radioactivity remained near the point of application (table 1). Attempts to extract the radioactivity from the urine, neutral or acidified, with various organic solvents—i.e., chloroform, carbon tetrachloride, diethyl ether, petroleum ether, benzene, and hexane—resulted in the removal of only trace amounts.

To determine if the radioactive phosphorus present in the urine was in the inorganic form, a portion of the composite sample was treated with "magnesia mixture" to precipitate the inorganic phosphates. This treatment removed only trace amounts of radioactivity. When aliquots of the 12-hour composite sample were chromatographed by the ethanol-hydrochloric acid system (fig. 4), only 16.8% (mean of 4 chromatograms) of the radioactivity ran with the R_f of, and coincided with, the color developed by the Hanes-Isherwood reagent of added dimethyl hydrogen phosphate and/or dimethyl hydrogen phosphite (R_f 0.47). Nearly 77% of the radioactivity ran with a mean R_f of 0.23. This unknown metabolite also gave color development (blue-green) when treated with the Hanes-Isherwood reagent.

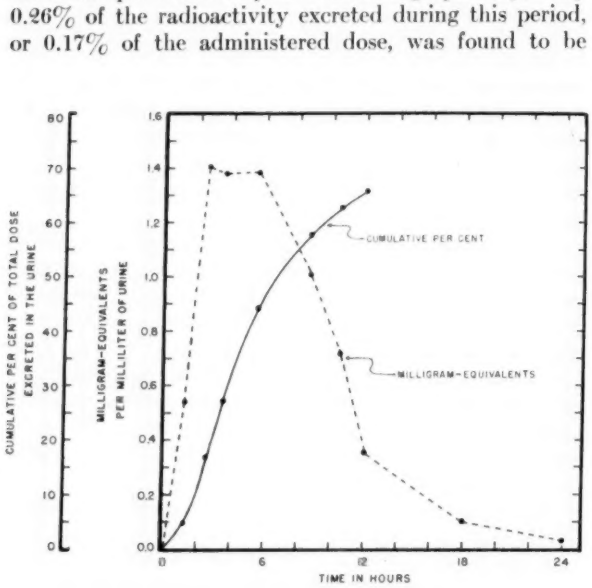
CUMULATIVE PER CENT OF TOTAL DOSE EXCRETED IN THE URINE
MILLIGRAM-EQUIVALENTS PER MILLILITER OF URINE
CUMULATIVE PER CENT
MILLIGRAM-EQUIVALENTS
TIME IN HOURS

FIG. 3.—Radioactivity in the urine of a cow following oral administration of P^{32}-Bayer L 13/59.

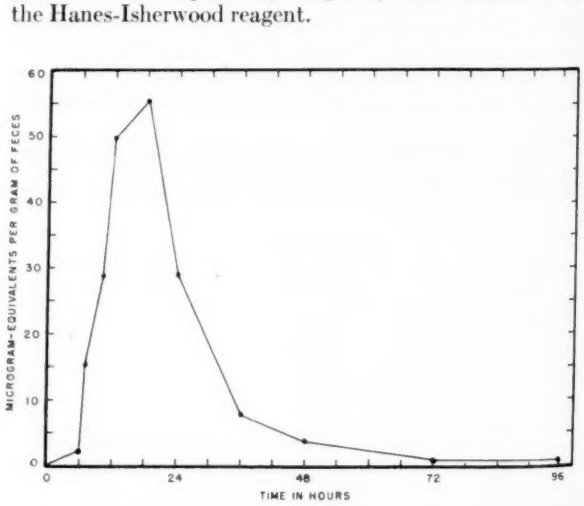

FIG. 5.—Radioactivity in the feces of a cow following oral administration of P^{32}-Bayer L 13/59.

Feces.—Trace amounts of radioactivity were first detected in the feces about 3 hours after treatment (fig. 5). There was a gradual increase until the peak was reached in the 18-hour sample, with an activity of 55.4 μg-equivalents/gram (wet weight). Less than 3% of the total dose was accounted for in all the fecal samples. No chromatographic analyses were performed on the fecal radioactivity.

DISCUSSION.—DuBois & Cotter (1955) state that the low mammalian toxicity of L 13/59 may be due to its rapid detoxification in animal tissues. Employing a bioassay procedure in which the anticholinesterase activity of the compound was used to detect its presence, these workers found that, when sublethal doses were administered to rats by intraperitoneal injection, the compound disappeared from the serum at a rapid rate, and only a small percentage was excreted unchanged in the urine. These postulations and findings are supported by the present research with a much larger mammal.

Following the rapid absorption and metabolism of L 13/59, the metabolic products are excreted in the urine in a pattern similar to that reported for several other organic phosphorus compounds (Jensen *et al.* 1952, Dahm 1952, March *et al.* 1955). Although the rapidity of the degradatory and excretory processes and the large percentage of the administered dose accounted for in the urine in such a brief period would likely limit the use of the compound as a chemotherapeutic agent, such characteristics would be desirable from the point of view of possible storage in tissues.

Only low levels of radioactivity were found in the grubs. The slow rates of increase and elimination and the lack of concurrence with blood levels of radioactivity in the cow suggest a metabolic pathway in these insects that differs from that of the host.

The fact that no DDVP was detected in the blood, milk, or urine does not eliminate the possibility of its being formed and rapidly exerting its toxic action and-or being metabolized.

Because of the small percentage of the excreted radioactivity that ran with the R_f of the probable metabolites, dimethyl hydrogen phosphate and dimethyl hydrogen phosphite, the possibility of these compounds being formed and undergoing further hydrolysis, either *in vivo* or after excretion in the urine, was investigated. The monomethyl esters of dimethyl hydrogen phosphate and dimethyl hydrogen phosphite were produced by hydrolysis of the parent compounds, and the R_f's were found to be 0.09 and 0.06, respectively, by the ethanol-hydrochloric acid system. The major metabolite did not have the R_f of either of these compounds or orthophosphoric acid, and only trace amounts of radioactivity were found at these R_f's (fig. 4). From these data it appears that the major metabolic pathway of L 13/59 in the cow is not by rupture of the P-C bond, but involves either the hydrolysis of the *O*-methyl ester linkage or modification of the 2,2,2-trichloro-1-hydroxyethylphosphonate portion of the molecule.

SUMMARY.—The metabolism and excretion of P^{32}-labeled Bayer L 13/59 in a lactating Hereford cow and its uptake by the cattle grub *Hypoderma bovis* (L.), were studied following oral administration of the compound to the animal at the rate of 25 mg./kg.

The peak of radioactivity in the blood was attained between the first and third hours after treatment, with a maximum of 15.1 μg-equivalents/ml. in the 2-hour sample. When the radioactivity in this sample was analyzed chromatographically, by the benzene-triethylene glycol system, only about 7.5%, or 1.1 μg./ml., ran with the R_f of L 13/59. There appeared to be a slower rate of dissipation of radioactivity in the grub exudate than in the blood.

Only low levels of radioactivity were detected in grubs removed at various intervals after treatment, and the maximum radioactivity per unit weight was found in those grubs removed 6 through 24 hours after treatment.

Less than 0.2% of the radioactivity representing the total dose administered was secreted in the milk at the end of 144 hours. When a 6–48 hour composite sample was extracted repeatedly with chloroform, less than 10% of the radioactivity behaved like unchanged L 13/59. When the chloroform extracts were analyzed chromatographically, only trace amounts of radioactivity ran with the same R_f as L 13/59. About 23% of the radioactivity present in the composite milk behaved like inorganic phosphorus. These findings were supported by bioassay studies with house flies.

L 13/59 was rapidly metabolized by the cow and eliminated via the urine. The peak of elimination was attained after $2\frac{1}{2}$–$5\frac{1}{2}$ hours with a concentration of about 1.4 mg-equivalents/ml. of urine. After 12 hours about 66% of the dose was accounted for in the urine. When the urine was analyzed chromatographically by the xylene-Carbowax and the benzene-triethylene glycol systems, only 0.26% of the radioactivity excreted during this period was unchanged L 13/59. Chromatography of the urine metabolites by the ethanol-hydrochloric acid system disclosed that only 16.8% of the radioactivity ran with the R_f of dimethyl hydrogen phosphate or dimethyl hydrogen phosphite. About 76%, an unknown metabolite, ran with a mean R_f of 0.23 and gave a color test by the Hanes-Isherwood (1949) technique. These data indicate that the major metabolic pathway of this phosphonate in the cow is not by rupture of the P-C bond. No DDVP was detected in the blood, milk, or urine.

Bayer L 13/59 was readily absorbed by the cow, as evidenced by the small amount of radioactivity in the feces. Less than 3% of the dose was accounted for in all fecal samples collected.

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Litter Treatment with Malathion to Control the Chicken Body Louse[1]

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There seems to be disagreement among poultrymen and scientific workers as to the exact nature and assessment of losses from infestations of poultry lice. There is no doubt however that heavy louse infestations irritate chickens and most poultrymen are desirous of ridding their flocks of lice, particularly when infestations are severe. Present methods of controlling lice on chickens include applying insecticidal dusts directly to the birds, both individually and collectively; by applying sprays to the roosts or poultry house and occasionally by dipping. One of the most frequently used methods is that described by Bishopp & Wood (1939) using sodium fluoride and known as the "pinch method."

Recently there has been some stimulus to investigate the possibilities of applying some of the newer organic insecticides as dusts to the litter to control chicken ectoparasites. Most chickens (hens more than roosters) dust themselves freely in dry litter, thus the reasoning behind this method appears to be sound. Vincent (1954) and Harding (1955) obtained good control of the northern fowl mite, *Bdellonyssus sylviarum* (C. and F.) on hens by applying the organic phosphate insecticide malathion as a 4% dust to the litter and nests. Fairchild & Dahm (1955) demonstrated very effective control of chicken lice on hens by applying aldrin, chlordane and lindane dusts to the litter of poultry houses.

At the University of Maryland Poultry Farm, tests were made during the winter and spring of 1955 to determine the effectiveness of this method of litter application in controlling natural infestations of the chicken body louse, *Menacanthus stramineus* (Nitz.) on White Rock and New Hampshire laying chickens. The insecticide used in the experiments was 4% malathion dust. Malathion was selected mainly because of its general effectiveness as an insecticide and its comparatively low mammalian and avian toxicity. All litter treatments included also mixing a small handful of the dust in the nesting material of each nest.

Since there was a possibility that eggs would come in contact with the insecticide, tests were carried out to determine the effect on hatchability of 4% malathion dust when applied to eggs. Residue analyses were also performed by applying 4% malathion dust to eggs and then making malathion residue analyses on the yolks and whites to determine whether or not internal contamination had taken place.

CHICKEN BODY LOUSE CONTROL.—*Procedure.*—Four pens each 400 square feet in area containing approximately 100 hens and 5 roosters per pen were included in the tests. The litter in each pen consisted of 100% woodshavings, very dry and built up to a depth of about 4 inches. Before treating, 10 hens and 3 roosters in each pen were caught at random, banded and the degree of louse infestation was given a numerical value as follows: 0 = no lice observed; 1 = light infestation; 2 = moderate infestation; 3 = heavy infestation with scaly skin. Mean louse infestations on the banded hens and roosters were determined. In general the louse infestations were located in the vent and breast regions on both hens and roosters, with roosters usually being more heavily infested than hens.

In pen 1, 4% malathion dust was applied by hand to the litter at the rate of 0.5 pound to 20 square feet. In

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Table 1.—Litter and nest treatments with 4% malathion dust against the chicken body louse on White Rock and New Hampshire Chickens.[a]

Pen	Rate of Application[b]	Mean Louse Infestation: Before Treatment	After Treatment (Days): 1	4	8	25	35	45
1	0.5	2.3	0.0	0.0	0.0	0.0	0.0	1.1
2	.25	1.6	0.0	0.0	0.0	0.0	0.0	1.3
3	.2	2.6	0.0	0.0	0.0	0.0	1.5	—
4	.1	2.1	0.0	0.0	0.0	1.1	—	—

[a] Records made from 10 hens and 3 roosters in each pen. Litter was dry in all pens.
[b] In pounds to 20 square feet.

addition, a small handful of malathion dust was mixed in the nesting material of each nest. The procedure was the same for the other three pens except the rate of application was decreased. In pen 2 the rate was 0.25 pound to 20 square feet; in pen 3 the rate was 0.2 pound to 20 square feet and in pen 4 it was 0.1 pound to 20 square feet.

Results.—To determine the effectiveness of the treatments the banded hens and roosters in each pen were examined at definite intervals and the mean louse infestations determined. Table 1 gives the results of the various treatments. Excellent initial control on both hens and roosters was obtained at all rates of application used. With the 0.5 pound and 0.25 pound treatments it was at least 5 weeks before nymphs of lice began to appear.

HATCHABILITY TESTS.—*Procedure.*—To determine the effect of 4% malathion dust on general hatchability, egg samples were rubbed with malathion using a 2-inch square of cheesecloth. Egg samples (100 per sample) were treated by the following methods: (1) malathion rubbed over the entire egg; (2) malathion rubbed over one-half of the egg (away from air cell); (3) sample of eggs collected 7 days after litter treatment of 0.25 pound to 20 square feet; (4) non-treated control. Immediately after treatment or collection from the litter treated pen the egg samples were incubated. The experiment was duplicated except in the case of the eggs from a litter-treated pen.

Results.—Table 2 gives the combined results of the two hatchability tests. As shown in the table there was no significant adverse effect from the treatments.

RESIDUE TESTS.—*Procedure.*—To determine whether malathion residues on eggs could possibly contaminate the whites and yolks of eggs, egg samples (12 per sample) were treated with 4% malathion dust by the following methods: (1) malathion rubbed over the entire egg, and then put under refrigeration for 2 weeks; (2) egg, cracked slightly by hand then malathion rubbed over the entire egg and put under refrigeration for 2 weeks; (3) malathion rubbed over the entire egg; (4) malathion rubbed over

Table 2.—The effect on hatchability of 4% malathion dust applied to eggs before incubation.

Test No.	Treatment	Number of: Eggs	Chicks	Infertile	Per Cent Hatch: Fertile Eggs	Total Eggs
1	One-half of egg[a]	200	159	17	86.8	70.9
2	All of egg	200	163	21	91.0	81.5
3	Eggs from treated pen[b]	100	74	10	82.2	74.0
4	Control	194	148	22	86.0	76.2

[a] Away from air cell.
[b] Pen treated at the rate of 0.25 lbs. to 20 square feet; eggs collected 1 week after treatment.

one-half of the egg (away from air cell); (5) and (6) samples collected 6 days after litter treatments of 0.5 pound and 0.25 pound to 20 square feet respectively; (7) untreated control. The time elapse between egg treatments and analysis for malathion varied from 4 weeks for the samples held under refrigeration to 2 weeks for all other samples.

Results.—The malathion residue analyses[2] on the samples submitted showed no malathion present (in p.p.m.) in the whites and yolks when compared with the control sample.

SUMMARY.—Litter treatments of 4% malathion dust applied at the rates of 0.5, 0.25, 0.2, and 0.1 pound to 20 square feet gave excellent initial control of the chicken body louse on White Rock and New Hampshire hens and roosters. The 0.5 and 0.25 pound treatments controlled lice for at least 5 weeks. All litter treatments were by hand and included mixing a small handful of the dust in the nesting material of each nest. The litter in all pens treated was dry giving ample opportunity for the chickens to dust themselves.

Tests (500 eggs) were carried out to determine the effect of 4% malathion dust on hatchability. In addition residue tests (72 eggs) were made to determine if malathion residues on eggs could contaminate the interior. No effect on hatchability was found and no malathion residues were detected in the samples submitted.

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[2] Acknowledgement is made to Rowland C. Brandenburg, Assistant Entomologist who performed the analyses.

Preparation of P^{32}-Labeled Bayer L 13/59 and DDVP, and Their Absorption and Translocation in the American Cockroach[1]

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Interest in organic phosphorus insecticides has been greatly stimulated because of their effectiveness and the slowness of insects to develop resistance to them. To aid in their toxicological studies Fernando *et al.* (1951), Lockau & Ludicke (1952), Jochum (1953), Dahm (1953) and others have used radioactive phosphorus.

Bayer L 13/59 (Barthel *et al.* 1954) and its dehydrochlorination product DDVP (Barthel *et al.* 1955, Mattson *et al.* 1955) have a high anticholinesterase activity (Babers & Mitlin 1955) and are toxic to several species of insects (von Rumker 1955). These materials have been labeled with phosphorus^{32} to aid workers in this Branch in their research on the systemic effects of L 13/59 in mammals, and to study their penetration, distribution, and metabolism in the American cockroach, *Periplaneta americana.* (L.).

EXPERIMENTAL.—Because the volatility of intermediates and the level of radioactivity employed in these syntheses were serious contamination hazards, an adequately shielded vacuum manifold (fig. 1) was used because it permitted transfers to be made in a closed system. The heating coil on the manifold was made from 24-gauge Nichrome wire and had a resistance of 15 ohms. Heat was controlled by the use of a variable transformer. This manifold is a modification of one described by Calvin *et al.* (1949).

At the start of the synthesis, drying tube *T1* was filled with calcium chloride and *T2* with a mixture of calcium chloride and solid sodium hydroxide. After *D* had been charged with 7.5 ml. of dry methanol and *H* with 3.5 ml. of dry chloral, *D*, *G*, and *H* were cooled with dry ice, *E* and *F* with ice-salt, and *W* with tap water. The manifold pressure was reduced to 1 mm.; *D*, *C*, and *H* were isolated; and the pressure in the remainder of the manifold was adjusted to that of the atmosphere.

PHOSPHORUS^{32} TRICHLORIDE.—This product was prepared in the usual manner adapted to manifold operation. After its Vycor shipping container had been opened with a device similar to that described by Coburn & Roan (1950), 1 gram of red phosphorus[3] containing 168 mc. of P^{32} was placed in *A* (fig. 1) together with 1.25 ml. of unlabeled phosphorus trichloride. After being heated slowly at first to prevent bumping, the mixture was refluxed vigorously and dry chlorine rapidly introduced through a large tube that barely dipped into the surface. When solid phosphorus pentachloride began to appear, heating was stopped, but the flow of chlorine was continued to prevent stoppage in the tube until the reaction mixture had cooled to room temperature. The flow of chlorine was then halted and the reaction flask cooled with dry ice.

Four grams of dry powdered antimony was then quickly added to the reaction mixture, the flask returned to the manifold, and the dry ice removed. The decomposition of the phosphorus pentachloride was initiated by heat from a small flame, controlled by intermittent cooling with ice, and finally completed by refluxing the reaction mixture until all the white solid had dissolved. The reaction mixture and *E* and *F* were then cooled with dry ice, and the excess chlorine was removed with the water pump. Finally the manifold pressure was reduced to 1 mm. and the manifold closed to the pump. The water was removed from the condenser jacket, *W*, and the cooling baths from *A*, *E*, and *F*, and the phosphorus^{32} trichloride transferred to *B*, previously cooled in dry ice. The total yield was 5.04 grams, 79% of theory.

P^{32}-DIMETHYL HYDROGEN PHOSPHITE.—The dry ice was shifted from *B* to *C* and, after the phosphorus^{32} trichloride had transferred, the manifold pressure was returned to normal. The dry ice was removed from *C* by a stream of air, *D* was cooled with an ice-salt bath supported on a magnetic stirrer, and traps *E* and *F* were cooled with ice-salt baths. The phosphorus^{32} trichloride was added to the methanol at 1 to 2 drops per second with vigorous stirring, which was continued for an additional 10 minutes.

The coil on the manifold was heated at 20 volts, and *D* was surrounded by a warm water bath. Traps *E* and *F* were cooled with dry ice, and most of the by-products were removed from the manifold with the water pump. In order to remove the remaining by-products, *D* was cooled with ice, *G* was emptied, and the system was pumped with the high-vacuum pump until frothing of the reaction mixture had ceased. Then, while the reaction mixture was gently refluxed, the manifold was alternately opened and closed to the pump until the pressure remained at 1 mm. The remainder of the manifold was isolated, *D* was heated at 65 volts, and the P^{32}-dimethyl hydrogen phosphite transferred to *B*, which was cooled with dry ice. The yield was 2.76 grams, 68.5% of the theory.

P^{32}-BAYER L 13/59.—Unit *C* was isolated from the manifold, the dry ice was removed from *H*, and 2.5 ml. of chloral was transferred to *B*. Units *H* and *B* were then isolated, the dry ice was removed from *B*, and when the reaction mixture had reached room temperature, the pressure in all units of the manifold was returned to normal.

Tube *B* was adequately shielded and removed from the manifold, and the contents were mixed with a glass rod. The tube was then closed with a glass stopper that also enclosed the rod, placed in a boiling-water bath, and allowed to remain for 8 hours on top of a warm steam bath. The reaction mixture was diluted with 5 ml. of carbon tetrachloride, cooled, seeded, and stirred occasionally. When crystallization was complete, the mother liquor was removed through a sintered-glass filter stick at reduced pressure. The crystalline P^{32}-L 13/59 was washed with 2 ml. of cold ether and sucked dry. The product weighed 3.25 grams, 27.1% of the theory based on the added phosphorus trichloride and red phosphorus^{32}, and its specific activity was 4,830 counts/minute/μg. determined in a windowless counter. Unlabeled L 13/59 prepared in a

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[3] Supplied by Atomic Energy Commission, Ltd., of Canada upon authorization of the U. S. Atomic Energy Commission.

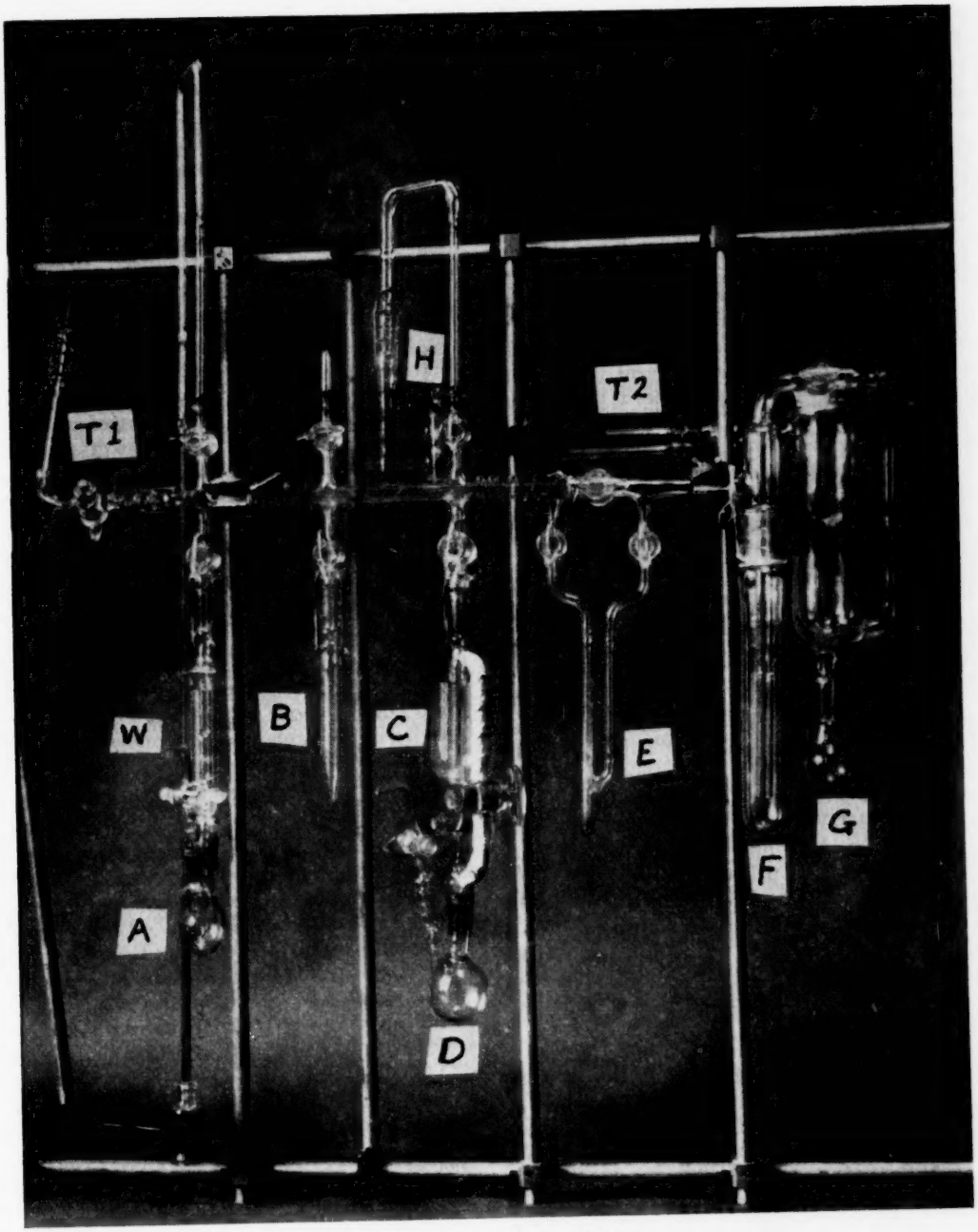

FIG. 1.—High-vacuum manifold.

similar manner melted at 74 to 75° C. and cleared at 78°.

Radiometric assay of a paper chromatogram of the product, developed in *n*-butanol saturated with water, indicated that it was 91% P^{32}-L 13/59. This value agrees with that of 90.1% independently determined by W. E. Robbins of this Branch (private communication).

P^{32}-DDVP.—Two hundred milligrams of P^{32}-L 13/59 were dissolved in 1.5 ml. of water and dehydrochlorinated at 40° C. by 2 drops of 40% sodium hydroxide (Barthel *et al.* (1955)). The DDVP was extracted with three 1-ml. portions of ether, and after being dried over Drierite the ether was evaporated. The product weighed 80 mg. (46.5% of theory) and had a specific activity of 812 c/m/μg. corrected for decay.

Radiometric assay of a paper chromatogram of the product, developed in *n*-butanol saturated with water, indicated that it contained 86% of P^{32}-DDVP.

PAPER CHROMATOGRAPHY OF P^{32}-L 13/59 AND DDVP.—Although P^{32}-L 13/59 and DDVP may be separated on paper from their impurities with *n*-butanol saturated with water, they both have R_f 0.87 in this solvent.

During the search for a solvent system suitable for the separation of these products from each other, radiometric assay of chromatograms developed in *n*-butanol (12):

pyridine (8): water (6)[4] showed them to have R_f values of 0.96 and 0.73, respectively. However, when the chromatograms were assayed for esterase inhibition by the method described by Cook (1955), the zone of L 13/59 gave a positive test but that of DDVP gave a negative test. Presumably the latter is changed to an unidentified noninhibiting product by the pyridine.

P^{32}-L 13/59 and DDVP were separated from each other and from their impurities in the following manner: The solvent, consisting of 25 ml. of Skellysolve B and 0.62 ml. of *n*-butanol saturated with water, was added directly to a 500-ml. graduated cylinder, where it was mixed and allowed to settle for 30 minutes. Two ½-inch blank strips of Whatman No. 1 paper were first run in the solvent to absorb the excess water, and then the solvent was used to develop chromatograms of 1-μg. quantities of L 13/59 and DDVP spotted on fresh strips.

Subsequent to the decay of the radioactivity below a useful level, Cook's anti-esterase test has been used for assay of the chromatograms. Chromatograms of P^{32}-L 13/59 and DDVP showed anti-esterase activity at R_f 0.0, 0.15, 0.7, and 0.85. Radiometric assay previously had indicated that L 13/59 and DDVP were located at R_f 0.7 and 0.85, respectively. This was confirmed by tests of toxicity to female house flies[5] made on zones that were sectioned from chromatograms of 5 μg. of P^{32}-L 13/59 and 2 μg. of P^{32}-DDVP developed on 1-inch strips in a similar manner. The zone at R_f 0.7 from L 13/59 killed 60% and the zone at R_f 0.85 from DDVP killed 80% of the flies. The presence of toxic material was not indicated in the other zones except R_f 0.15 from DDVP, which killed 20%. This indicated the presence in P^{32}-DDVP, and also in purified unlabeled DDVP which behaved similarly, of an impurity that possesses a high order of toxicity both to pseudocholinesterase and to house flies.

The zones at R_f 0.85 from P^{32}-L 13/59 and at R_f 0.7 from DDVP are assumed to be trace quantities of DDVP and L 13/59. The zones at R_f 0.0 from both substances possessed strong anti-esterase activity, and gave positive tests for unsaturation when sprayed with alkaline permanganate.

ABSORPTION AND TRANSLOCATION OF P^{32}-L 13/59.—With the device described by Roan & Maeda (1953), 1.205 μl. of freshly prepared solutions of P^{32}-L 13/59 in absolute ethanol was applied to the dorsal cervical membrane of last-instar American cockroach nymphs. At varying time intervals the end of an antenna was clipped and a drop (about 1 μl.) of hemolymph was caught in an aluminum counting cup. Because of the volatility of L 13/59, 1 mg. of unlabeled material in acetone was added to the counting cup. The radioactivity was determined after the solvent had been evaporated at room temperature.

After treatment with 29 μg. of L 13/59, the hemolymph was highly radioactive within 15 minutes and remained radioactive for more than 6 hours. After 6 hours the cockroaches were prostrate and barely alive. Although one cockroach bled continuously, the blood of the other prostrate treated insects clotted normally. One cockroach was dissected at 20 and another at 48 hours after treatment with 23.3 μg. of L 13/59, and the radioactivity was determined in various tissues. The results are shown in table 1.

Table 1.—Radioactivity[a] in tissues of American cockroach nymphs treated with 23.2 μg. of P^{32}-L 13/59.

Tissue	After 20 Hours		After 48 Hours	
	c/m	μg.-Equivalent	c/m	μg.-Equivalent
Heart	184	0.04	94	0.02
Fat body	130	.03	358	.07
Esophagus	310	.06	566	.12
Crop	5,063	1.05	12,245	2.54
Proventriculus	5,563	1.15	3,882	.81
Ventriculus	9,414	1.95	7,717	1.60
Ileum	365	.08	217	.05
Colon	1,045	.22	2,825	.59
Malpighian tubules	266	.06	165	.03
Ventral nerve cord	374	.08	38	.01
Brain	68	.01	0	—
Cast skin[b]	—	—	816	.17
External wash	23,148	4.80	—	—
Total recovered		9.53		6.01

[a] Corrected for decay.
[b] From an insect that molted about 26 hours after treatment.

ABSORPTION AND TRANSLOCATION OF P^{32}-DDVP.—Last-instar American cockroach nymphs were treated with 1.205 μg. of P^{32}-DDVP in 1.205 μl. of absolute ethanol, as previously described for L 13/59. After 5 minutes, and at regular intervals thereafter, the radioactivity of the hemolymph was determined as described above, but it did not rise above background within 2 hours. Radioactivity in the tissues was determined after 2, 4, and 22 hours as described above and the results are shown in table 2.

Table 2.—Radioactivity[a] in tissues of American cockroach nymphs treated with 1.205 μg. of P^{32}-DDVP.

Tissue	After 2 Hours c/m	After 4 Hours c/m	After 22 Hours c/m
Brain	69	—	22
Ventral nerve cord	0	—	29
Heart	551	28	7
Esophagus	28	—	—
Crop	221	—	—
Proventriculus	226	—	65
Ventriculus	91	—	—
Ileum	239	—	—
Colon	0	180	—
Malpighian	0	—	41
Fat body	0	54	376

[a] Corrected for decay.

Several cockroaches were treated with DDVP and immobilized by a rubber dam or plastic tape in an attempt to collect feces. After 24 hours no defecation had occurred and the filter paper upon which the insects had been placed was not radioactive.

One cockroach held overnight was dead the next morning, but had regurgitated an appreciable quantity of highly radioactive material. This finding was similar to that reported by Jochum (1953) following the treatment of several species of insects with P^{32}-parathion.

PRODUCTS OF METABOLISM.—To gain some knowledge

[4] Solvent suggested by B. H. Alexander of this Branch.
[5] These tests were made by B. A. Butt of this Branch.

of the metabolites, extracts of various tissues taken from insects treated with the P32-L 13/59 were chromatographed.

The extirpated wet tissue was ground with 1 gram of anhydrous silicic acid and placed in a small envelope made of Whatman No. 1 filter paper. The envelope was then stapled to a long strip of 1-inch Whatman No. 1 chromatographic paper, and the tissue was extracted by the technique described by Winteringham (1953). The following solvents were used: absolute ethanol, 95% ethanol, acetone, ether, and ethanol made 70% with 5% aqueous ammonia. All radioactivity was removed from the fat body and the foregut with the alcohol-ammonia mixture, but none of the solvents removed all of the activity from the mid- and hind-guts.

After the extraction and concentration onto the strips, they were chromatographed with the solvent mixture *n*-butanol (12): pyridine (8): water (6). The developed strips were cut into 1-inch sections, and the radioactivity was determined. From the fat body of cockroaches treated 20 hours previously, zones of radioactivity were found at R_f 0.73 and 0.96, and some activity was present throughout the strip. From the fore and mid guts most of the activity corresponded to R_f 0.73. These zones corresponded to those obtained when DDVP and L 13/59 were chromatographed under similar conditions. However, hydrolysis by the pyridine might have occurred and the results were not acceptable. By the time an adequate chromatographic procedure had been developed for the separation and identification of L 13/59 and DDVP, the radioactivity had fallen below workable levels and none of the metabolites were identified.

Following completion of the experiments with P32-labeled materials described above, Robbins *et al.* (1956-A) reported on metabolites at R_f 0.23 and 0.47 developed with 95% ethanol (7.5): 0.2% v/v hydrochloric acid (2.5) in the urine of a cow following oral treatment with P32-L 13/59. Their chromatographic methods were employed on whole guts from cockroachs following topical application of unlabeled L 13/59 and from control cockroaches. In this laboratory chromatograms of treated cow urine, kindly furnished by Robbins, exactly duplicated those described by them when developed in 95% ethanol (7): 0.2% v/v hydrochloric acid (3). Poor resolution was obtained when the solvent contained hydrochloric acid prepared on a weight-volume basis.

Good chromatograms of cockroach material were obtained with extracts that were made with solvent composed of 40% trichloroacetic acid (2): water (2): 95% ethanol (8) and developed with 95% ethanol (7.5): 0.2% v/v hydrochloric acid (2.5). In this solvent the major "cow-metabolite" had R_f 0.16 instead of 0.23, but when it was eluted and run in the presence of cockroach extract it traveled with R_f 0.24 in the zone of nucleic acid or nucleotide located at R_f 0.28. Phosphoric acid (R_f 0.20) in the presence of cockroach extract traveled with the zone of nucleic acid or nucleotide at R_f 0.47. The minor "cow metabolite," dimethyl hydrogen phosphate, traveled with R_f 0.55 instead of 0.47 both alone and in the presence of cockroach extract.

The solvent pyridine (8): conc. ammonium hydroxide (2) described by Robbins *et al.* (1956b) for the separation of diethyl hydrogen phosphothioate and diethyl hydrogen phosphate was found to give a good separation of dimethyl hydrogen phosphate (R_f 0.31) from these acids and from the cockroach extractives (R_f 0.0). The chromatograms were passed several times through a jet of steam before color development in order to eliminate background color.

Because of the quantity of dimethyl hydrogen phosphate (10 μg.) required for the detection of phosphorus (Hanes & Isherwood 1949), it is evident that the metabolites can best be detected when they are derived from P32-L 13/59. The occurrence of the "cow metabolites" in cockroaches treated with unlabeled L 13/59 has not been verified. No evidence of unchanged L 13/59 was found on chromatograms tested for esterase inhibition as described above.

DISCUSSION.—Bayer L 13/59 and DDVP are rapidly absorbed through the cervical membrane of the cockroach. After 20 hours all tissues examined from L 13/59 treated insects were radioactive but most of the internal radioactivity was present in the intestinal tract. Only 4.73 μg-equivalents of L 13/59 were accounted for internally, although 23 μg. had been applied, but a large amount of the material was not absorbed during 20 hours as shown by the high radioactivity of the external ethanol wash.

High radioactivity in the crop may indicate that under certain conditions this organ functions as part of the excretory or detoxication system, as suggested by Fernando *et al.* (1951). In their experiments several radioactive phosphorus insecticides (or radioactive metabolites) were concentrated in the fore gut within a few minutes after the insecticide was applied to the cervical membrane. Zeid *et al.* (1953) also noted high radioactivity in the fore gut of cockroaches treated with radioactive pyrethrins. It has been widely accepted (Waterhouse & Day 1953) that the fore gut serves merely as a passage or storage place for food and that the mid gut is the main site of absorption. Jochum (1953) reported that in parathion-poisoned insects the hemolymph was absorbed into the gut and that death was delayed when the hemolymph level was mechanically maintained.

In none of these experiments has it been demonstrated that the absorption was directly into the fore gut. However this may have occurred because the cervical membrane is near the fore gut, and soon after penetration of the integument the concentration of material would probably be high, momentarily at least, in the vicinity of the fore gut. It is also possible that the radioactivity in the fore gut was due to material that had been absorbed in the mid gut and then passed anteriorly through the stomodeal valve.

Because of its greater toxicity, much smaller doses of DDVP than of L 13/59 were applied. The inability to detect radioactivity in the hemolymph was probably due to the low specific activity of the DDVP, the small amounts involved, and the rapid deposition of DDVP in the tissues. Within 2 hours no radioactivity could be removed from the outside of the cockroach by washing with alcohol or water and many of the tissues were radioactive. The heart was especially radioactive. Four hours after treatment the colon showed considerable activity, the activity in the heart was greatly decreased, and some activity was present in the fat body. Only about three-fourths the ra-

dioactivity of the applied DDVP was accounted for after 22 hours; most of it was in the fat body and a small quantity was in the gut. Because the DDVP-treated cockroaches did not defecate, the fate of the DDVP is not clear, although because of its volatility, some could well have been eliminated through the spiracles.

Because the phosphorus insecticides usually inhibit cholinesterase *in vitro* and because the level of the enzyme in poisoned insects may be very low, it has been assumed that the inhibition of the enzyme explains the mode of actions of the insecticides (Metcalf 1955). However, the role of the enzyme in insects is by no means established. It seems improbable that all phosphorus insecticides act only by inhibiting cholinesterase, since there is wide divergence in their distribution through the insect tissues. It seems more probable that several other physiological systems may be involved.

SUMMARY.—Radioactive Bayer L 13/59 was prepared from red phosphorus^{32}, and DDVP by dehydrochlorination of this L 13/59. Both insecticides were readily absorbed through the cervical membrane by the American cockroach, *Periplaneta americana* (L.). The distribution of L 13/59 was widespread, the hemolymph and all tissues being radioactive. After 20 hours most of the radioactivity was in the gut. The hemolymph of the DDVP-treated insects did not become demonstrably radioactive and many tissues were only slightly so. Much activity was concentrated in the heart, but after 22 hours most of the activity was in the fat body and only a small quantity in the gut.

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A Laboratory Method for Testing Grain Protectants Against the Grain Mite[1]

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A series of laboratory experiments were conducted in 1954 in order to determine a method for measuring the protective effect of various toxic dusts when used against the grain mite, *Acarus siro* L. In an earlier series of tests using treated wheat as the substrate, and the mortality of mites placed on this substrate as an indication of the protective qualities of the toxic material, it was found that a great amount of deviation resulted due to the large numbers of variables in the design. One of the major manifestations of variation was noted in the recovery of the treated mites. During the course of later investigations, a method was devised whereby the protective qualities of the dusts could be measured by the extent of penetration of the mites through treated flour. The method allowed the observation to be made *in situ*, thus obviating the necessity for removal and subsequent counting of live mites.

As pointed out by Winburn (1952), dust protectants are designed to prevent grain infestation rather than to eradicate infestations already present. Protectants act as contact poisons, not as fumigants. The design of the experiment described herein, therefore, was such as to measure the barrier effect of the protectants used rather than their effects as eradicants.

The following materials were used:

0.5% lindane in wheat dust.
10% methoxychlor in wheat dust.
1.1% technical piperonyl butoxide +0.08% pyrethrins in wheat dust.[3]
Ryania.[4]
Malathion, 25% (wettable powder).
Chlorthion, 25% (wettable powder).
Neotran
Bayer L 13/59.
10% piperonyl butoxide +1.0% pyrethrins.[5]

PROCEDURE.—Since *A. siro* leaves a noticeable trail behind it as it moves through flour, it was decided to set up the tests in test tubes, through the walls of which the extent of penetration into the treated flour could be observed and measured. A preliminary test using five 16-×150-mm. test tubes was set up in the following manner. A 1″×1″ square of water saturated fiber paper was placed in the bottom of each tube. Clean white whole wheat flour was sifted gently into the tubes to a depth of 52½ mm. Approximately 300 *A. siro* were introduced into each test tube with a fine camel's-hair brush. A ring of petrolatum was placed an inch above the flour surface to prevent the mites from leaving the tube. The tubes were then placed in a rack, where daily observation of the extent of penetration could be carried out with the least amount of disturbance to the tubes. The temperature of the holding room during the observations was 22° C. ±2°. Because of the limited exposed surface of the flour and the consequent lack of excessive moisture loss through surface evaporation, no attempt was made to control room humidity. The moisture content of the flour at any given depth, therefore, was determined mainly by the moisture gradient resulting through the use of the water saturated fiber paper in the bottom of each tube.

Within an hour after the introduction of the mites into the test tubes, a considerable amount of penetration into the first few millimeters of flour was observed. After 11 days, the pattern of mite trails in all five replicates ramified throughout the flour samples.

The tests proper were run in two series with each protectant being replicated six times. Six untreated checks, identical to those described above, were included in each series. The treated replicates were similar to the checks in design, except that the top 12½ mm. of flour was mixed with enough protectant to make up a dosage of 105 pounds of protectant mixture per 1000 bushels, or 7.94 grams/pound of wheat. This rather high dosage was chosen so as to increase the possibility of obtaining high positive results with at least one of the materials tested.

After the introduction of the mites into the test tubes, the tubes were assigned positions in the holding rack, each series occupying a randomized 36-slot square.

An index of activity was devised for the purpose of measuring the differences in general movement of the mites in each test tube. Numbers from one to five were assigned to indicate the relative degrees of penetration (fig. 1).

1. Complete pattern of mite trails throughout treated and untreated flour.
2. Complete pattern throughout treated flour with some movement into untreated flour.
3. Complete pattern only in treated flour with little or no penetration into untreated flour.
4. Sparse pattern in treated flour only.
5. At most, minute penetration into surface of treated flour.

After a holding period of 11 days, the test tubes were examined and indexed. Results are shown in table 1. An analysis of variance was carried out on each series to determine the significance of the results. The findings of these analyses are discussed below.

RESULTS.—In both series of tests, the analyses showed a high degree of significance between differences in treatments, but none between blocks. The results indicate that no part of either experimental block was subjected to unfavorable variation in temperature, light or moisture.

On the basis of the index method described above, the protectants were evaluated and compared. In the first series of tests, it was found that only lindane and pyrenone

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[2] The author wishes to acknowledge the assistance of Dr. H. H. Schwardt, Cornell University, Ithaca, N. Y., under whose direction this work was carried out.

[3] Pyrenone Wheat Protectant. Manufactured by U. S. Industrial Chemicals Co., New York, N. Y.

[4] Ryanicide 100. Manufactured by S. B. Penick and Co., New York, N. Y.

[5] "T-560." Manufactured by U. S. Industrial Chemicals Co., New York N. Y.

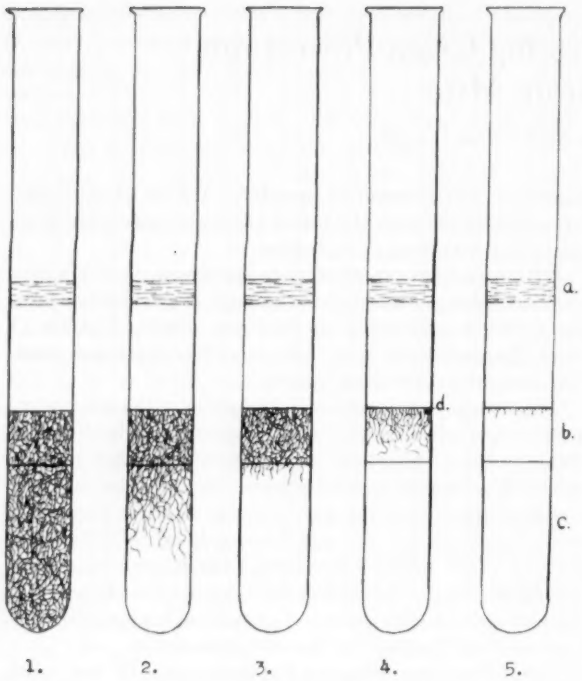

FIG. 1.—Test tubes showing various degrees of penetration by *A. siro* through treated and untreated flour. a. Vaseline barrier. b. Zone of treated flour. c. Zone of untreated flour. d. Mite trails.

1–10 concentrate were significantly better than the check at the 99% level of probability, while ryania proved better than the check only in 90 cases out of 100. Both lindane and pyrenone 1–10 concentrate were more effective than Pyrenone Wheat Protectant, methoxychlor, or ryania at the 99% level of probability, with lindane being somewhat more effective than pyrenone 1–10 concentrate in 99 cases out of 100. In the second series of tests, all materials were, on the basis of the analysis, better than the check at the 99% level of probability. Neotran appeared to be the best material, and was significantly better than lindane at the 99% level. There was no measurable difference between lindane and malathion. Both of these materials proved significantly more effective than Bayer L 13/59 at the 99% level. Lindane and malathion also showed a significantly greater effect as barriers than did Chlorthion, with lindane being better than Chlorthion in 99 cases out of 100 and malathion showing a better barrier effect in 95 cases out of 100.

Table 1.—Results of tests with wheat protectants using test tube index method.

Material	Replicates 1	2	3	4	5	6	Mean
Series 1							
Pyrenone Wheat Protectant	1	1	1	2	1	2	1.33
Pyrenone 1-10 concentrate	3	3	4	3	4	2	3.16
Lindane	3	4	4	5	5	4	4.16
Methoxychlor	2	1	1	1	2	1	1.33
Ryanicide 100	2	2	2	1	1	2	1.60
Check	1	1	1	1	1	1	1.00
Series 2							
Lindane	3	4	5	3	4	4	3.83
Chlorthion	3	2	3	3	3	3	2.82
Malathion	4	4	3	3	4	4	3.66
Neotran	4	4	4	5	5	5	4.50
Bayer L 13/59	3	3	3	2	2	2	2.50
Check	1	1	1	2	1	1	1.16

An examination of the test tubes 1 month after initiation of the tests showed no further activity except where mites had succeeded in reaching the untreated flour (index numbers 1, 2, and 3). In tubes with an index value of one, continued activity and reproduction in the untreated flour caused some increase in numbers of observed mites trails. In tubes with an index value of two or three, activity and reproduction in the untreated flour was limited, the increase in the number of visibile mite trails in any given tube rarely being great enough to change the index value of the tube involved.

CONCLUSIONS.—The test tube index method of evaluating protectants against the grain mite, *Acarus siro* L., appears to be highly effective and should prove to be a useful tool in future work on protectant materials. With minor variations, the method may be applicable for measuring the effectiveness of protectants against other arthropods in stored products as well.

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Translocation of BHC from High-Dosage Soil Treatments Applied Before Planting[1]

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The chlorinated hydrocarbon insecticides have attracted little attention as systemic insecticides, owing to their very low solubility in water and other physical and chemical properties. BHC and lindane are the exceptions to this generalization, since they have been credited with some systemic action by certain authors.

The first positive evidence that BHC in the soil can be translocated to the aerial portions of plants apparently was the work of Starnes (1950). He reported that both aqueous suspensions and benzene extracts of potato foliage from plants grown on soil treated with 10 pounds of gamma isomer of BHC per acre were highly toxic to *Aëdes* mosquito larvae. However Anderson (1955) reported that BHC was "not found to be effective as a root-absorbed systemic poison" in cotton.

Recently Wollerman (1955) found that soil applications of BHC, lindane, and alpha-beta cake (a by-product when lindane is made from BHC) are all systemic in trees, on the basis of both insect and daphnid tests. In another recent paper Chisholm *et al.* (1955) reported translocation of BHC in potatoes, carrots, and onions. Also at the national meeting of the Entomological Society of America, where our findings were presented orally in December of 1955, R. G. Haines at the same session reported on work at Rutgers University showing the translocation of lindane in corn plants grown in treated water culture solutions.

This report, a phase of a larger and more comprehensive long-time investigation, deals with the uptake and translocation of lindane and BHC from the soil by a number of agricultural crops, as revealed by chemical analyses of the harvested seed. Also the chemical work has been supplemented by certain biological and related tests.

Procedures and Results.—In the spring of 1952 a replicated field experiment was initiated at Ames, Iowa, in which 10 insecticides were applied to the soil at roughly 50 times the estimated maximum annual levels at which they might commonly be used commercially. Included was lindane at 50 pounds per acre. Applications were made by knapsack sprayers on 25′×29′ plots. The soil was fitted for planting before spraying in early June, and was disked soon after the insecticides were applied. The whole experimental area was planted to Pioneer 352 yellow dent field corn and Hawkeye soybeans in alternating pairs of rows on June 16.

This test plot was replanted to the same crops in the same arrangement in 1953. It was fitted with a 7-foot rototiller instead of plowing and disking, so as to minimize mixing along the plot margins. Normal germination, stands, growth, and yields were obtained from both crops in both years following all insecticide treatments. Each sample of soil for residue analysis consisted of 50 cores, 1 inch in diameter and 6 inches deep.

Most of the soybeans in four of the five lindane-treated replicates were removed early in 1953, and these rows were replanted with lima beans, Great Northern beans, green pod beans and yellow wax beans, respectively (two replicates of each). In 1954 the entire experimental area was again fitted with a rototiller and seeded to birdsfoot trefoil. However, two of the lindane-treated replicates were re-worked and planted to soybeans, Great Northern beans, lima beans, sunflowers, sweet corn and grain sorghum, one row of each crop in each plot.

In 1955 the two lindane-treated replicates were again rototilled and planted to two single-cross dent corn hybrids, two commercial sweet corn hybrids, Minhybrid 250 popcorn, Norghum grain sorghum, Hawkeye soybeans and sunflowers. Also duplicate plots in the same field were treated with 10 pounds per acre of lindane and gamma isomer of BHC, respectively, for additional plantings. These insecticides were applied in emulsion form with hand sprayers, and the plots were thoroughly disked immediately afterward. The crops listed above were also planted on these plots, along with Purdue 31 popcorn, Hawkeye soybeans, Henderson bush lima beans, Great Northern beans, green pod beans and yellow wax beans.

Samples were submitted to the Vincennes, Ind., laboratory of the Entomology Research Branch, U.S. Dept. of Agriculture, for lindane analyses all 4 years. They were as follows: *1952*—Shelled corn and hand-threshed soybeans, each from three replicate plots treated with lindane, plus appropriate controls. *1953*—Hand-threshed and cleaned samples of soybeans, lima beans, Great Northern beans, green pod beans and yellow wax beans, each from two replicate plots, plus appropriate controls from untreated plots. Also soil and shelled corn samples from four lindane-treated replicates were submitted at the end of the 1953 growing season, along with appropriate untreated control samples. *1954*—Hand-threshed and cleaned samples of soybeans, Great Northern beans, lima beans, sunflower, sweet corn and grain sorghum from two replicate plots treated with lindane, plus appropriate untreated control samples. Great Northern beans harvested from plants grown on clean soil using seed of the 1953 crop containing 0.13 p.p.m. of BHC were submitted in 1954; also a second set of soil samples collected early in the growing season. *1955*—Hand-threshed and cleaned samples of all the crops listed above were obtained, along with appropriate control samples of the same crops grown on untreated soil. Also soil samples were collected from all of the treated plots plus untreated areas at the end of the growing season.

All of the chemical analyses were made at the Vincennes laboratory, using the Schechter & Hornstein (1952) method. In this procedure all of the isomers of BHC are dechlorinated, the benzene recovered and nitrated, and the m-dinitrobenzene determined colorimetrically. The analytical method used determined all of the isomers of BHC and is sensitive to as little as 2 to 3 micrograms of BHC. The standard procedure for preparing the samples consisted first of a rapid chloroform wash in a funnel to

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remove any possible external contamination. Next they were dried and ground to fine powders in a hammer mill. A 100-gram subsample of each lot was then extracted with chloroform, and the extract analyzed for BHC as described above, using a Coleman spectrophotometer. In each analysis samples of the same crops, grown on untreated soil in adjacent areas, were employed as checks.

Table 1 summarizes the results obtained from the crops grown on plots treated with 50 pounds per acre of lindane in 1952 during all four growing seasons. The results from the lindane- and BHC-treated plots established in the spring of 1955 are presented in table 2.

BIOLOGICAL AND OTHER TESTS.—*Effects of Corn on Confused Flour Beetles.*—A preliminary experiment was carried out with confused flour beetle, *Tribolium confusum* Duval, adults fed on the dent and sweet corn samples grown in 1955. This experiment was started by putting five crushed kernels of corn and five active beetles from a

Table 1.—Results of chemical analyses of seed from plants grown at Ames, Iowa, on soil treated with 50 pounds per acre of lindane in spring of 1952.

YEAR	CROP AND VARIETY	REPLICATE NUMBER	BHC IN p.p.m.: Replicate	Average
1952	Hawkeye soybeans	1	0.93	
		2	0.88	
		3	0.40	0.74
	Pioneer 352 field corn	1	0	
		2	0	
		3	0	0
1953	Hawkeye soybeans	1	0.62	
		2	1.16	0.89
	Pioneer 352 field corn	1	0	
		2	<.01	
		3	0	
		4	<.01	<.01
	Henderson bush lima beans	1	0.27	
		2	0.11	0.19
	Great Northern beans	1	0.13	
		2	0.21	0.17
	Top notch golden wax beans	1	0.27	
		2	0.17	0.22
	Top crop green pod beans	1	0.31	
		2	0.24	0.28
	Lindane-treated soil (October)	1	4.8	
		2	5.1	
		3	5.0	
		4	4.4	4.8
1954	Hawkeye soybeans	1	0.27	
		2	0.27	0.27
	Iochief sweet corn	1	0.54	
		2	0.88	0.71
	Norghum grain sorghum	1	0.55	
		2	0.19	0.37
	Sunflower	1	0.22	
		2	<0.01	0.11
	Great Northern beans	1	0.06	
		2	—	—
	Henderson bush lima beans	1	<0.01	
		2	<0.01	<0.01
	Lindane-treated soil (June)	1	3.3	
		2	3.2	3.25
	Great Northern beans grown on clean soil from seed containing 0.13 p.p.m. of lindane.	1	<0.01	
		2	<0.01	<0.01
1955	Hawkeye soybeans	1	1.21	—
	GN2×LF5 dent corn	1	.33	
		2	.16	.25
	AE1×B44M1 dent corn	1	.23	
		2	.33	.28
	Iochief sweet corn	1	.43	
		2	.39	.41
	Tendermost sweet corn	1	.07	
		2	0	.04
	Minhybrid 250 popcorn	1	0	
		2	0	0
	Norghum grain sorghum	1	.14	—
	Sunflower	1	.45	—
	Lindane-treated soil (October)	1	3.50	
		2	3.20	3.35

Table 2.—Results of chemical analyses of seed from plants grown on soil treated with 10 pounds per acre of lindane or gamma isomer of BHC just before planting at Ames, Iowa.—1955.

INSECTICIDE	CROP AND VARIETY	REPLICATE NUMBER	BHC IN p.p.m. Replicate	Average
Lindane	GN2×LF5 dent corn	1	.26	
		2	.36	.31
BHC	GN2×L5F dent corn	1	0	
		2	0	0
Lindane	AE1×B44M1 dent corn	1	.29	
		2	.59	.44
BHC	AE1×B44M1 dent corn	1	.05	
		2	.32	.19
Lindane	Iochief sweet corn	1	.25	
		2	.27	.31
BHC	Iochief sweet corn	1	0	
		2	.05	.03
Lindane	Tendermost sweet corn	1	.26	
		2	.15	.21
BHC	Tendermost sweet corn	1	.08	
		2	.02	.05
Lindane	Minhybrid 250 popcorn	1	.02	
		2	0	.01
BHC	Minhybrid 250 popcorn	1	0	
		2	.09	.05
Lindane	Purdue 31 popcorn	1	.25	
		2	0	.13
BHC	Purdue 31 popcorn	1	.02	
		2	0	.01
Lindane	Hawkeye soybeans	1	.82	—
BHC	Hawkeye soybeans	1	.97	—
Lindane	Henderson bush lima beans	1	0	—
BHC	Henderson bush lima beans	1	0	—
Lindane	Great Northern beans	1	0	—
BHC	Great Northern beans	1	0	—
Lindane	Wax and green beans combined	1	.04	—
BHC	Wax and green beans combined	1	.06	—
Lindane	Norghum grain sorghum	1	0	—
BHC	Norghum grain sorghum	1	0	—
Lindane	Sunflower	1	.23	—
BHC	Sunflower	1	0	—
Lindane	Soil (October)	1	2.18	
		2	2.50	2.34
BHC	Soil (October)	1	4.12	
		2	3.68	3.90

stock culture in each of a series of petri dishes. These dishes were kept at room temperature and observed every other day for 90 days, beginning on December 10, 1955.

The general trend of the results showed that the crushed sweet corn kernels were relatively poor as a food medium for this insect, but that the soil treatments where the corn was grown apparently had no influence on beetle survival. Thirteen out of the 16 dishes containing sweet corn, including 3 of the 4 untreated control samples, contained 5 dead beetles long before 90 days had passed. Of the 3 remaining dishes provided with sweet corn as the only source of food 2, including the remaining control dish, contained only 1 live beetle at the end of 90 days.

The dent corn samples proved to be favorable media for beetle survival, regardless of the use of soil treatments on the plots where they were grown. At the end of 90 days 6 out of 23 dishes showed all 5 beetles still alive and active, 7 showed 4 alive, 6 showed 3, 1 showed 2, 2 showed 1, and only 1 contained 5 dead beetles. Of the 5 untreated dent corn controls included in the above total (i.e., those grown on untreated soil) 1 showed 2 dead, 3 showed 1 dead in each, and only 1 showed all 5 beetles still alive.

Germination of Corn and Soybeans.—When both corn and soybeans grew and yielded normally following the very excessive applications of soil insecticides made in 1952, we decided to have official germination tests run on replicated samples of both crops. These tests were made in the Iowa State College Seed Testing Laboratory in November, 1952.

These germination tests failed to show any tangible evidence of adverse effects from lindane or any of the other nine insecticides included in the experiment. The germination percentages for soybeans were 94, 96, 93, 95 and 94 for the untreated control samples, while the corresponding values for seed produced on plots treated with 50 pounds per acre of lindane before planting were 90, 94, 92, 90 and 95, respectively. The corn germinations were all somewhat low because of an early frost, but again there were only small differences between samples from the control and the lindane-treated plots, respectively. The germination percentages for four replicates of the former were 80, 92, 88 and 86, and of the latter 86, 80, 81 and 84.

Popcorn Quality.—Recently a question arose in industry regarding possible adverse effects on the popping expansion of popcorn produced on plants grown from lindane-treated seed. In order to get information bearing on this point Professors J. C. Eldredge and W. I. Thomas of the Iowa State College Agronomy Department volunteered to run popping tests on the remnants from our 1955 popcorn plots.

These samples included Minhybrid 250 grown on the plots treated in 1952 and 1955, respectively, and Purdue 31 grown on the plots treated in 1955. Minhybrid 250 showed popping expansions of 29.0 and 31.4 for the two untreated control samples, and 32.4, 32.0, 29.8, 30.2, 34.0 and 32.2 for the six samples harvested from plants grown on treated soil. Corresponding values for Purdue 31 were 30.1 and 33.0 for the control samples, and 31.0, 31.6, 28.6 and 30.4 respectively, for the samples from plants grown on treated soil. All of these values are considered normal for these particular hybrids.

Taste and smell tests were made on both freshly-popped and cooled samples from all 14 popcorn plots by two experienced popcorn tasters (Eldredge and Thomas) and one amateur (Lilly). No indications of off-flavor or abnormal odors were detected in any of the samples. In a further test a group of 12 people in a non-critical preference test unanimously indicated a choice of popped Minhybrid 250 from the plots treated with 10 pounds per acre of gamma BHC over the same variety grown on the untreated control plots.

SUMMARY AND CONCLUSIONS.—Fifty pounds per acre of lindane applied to the soil in 1952 resulted in low but definite amounts of BHC in the seeds of various crops the year of treatment and in the following three growing seasons. Also some of the crops grown in 1955 on plots treated just before planting with lindane and gamma isomer of BHC respectively at 10 pounds per acre showed small amounts of insecticide in the seed. This confirms previously published reports that plants can translocate these insecticides.

BHC occurred in the seed of several crops and varieties, which represented three plant families and included both low-oil and high-oil content seeds.

Soybeans were the one crop tested all 4 years from the plots treated in 1952. The averages for the various samples of this crop were 0.74, 0.89, 0.27 and 1.21 p.p.m. of BHC in 1952, 1953, 1954 and 1955, respectively.

The results obtained with dent corn were less consistent than those obtained with the other crops investigated. No trace of insecticide was found in three samples of field corn grown in 1952, and only two of four samples analyzed from the 1953 crop showed even weakly positive tests. However, 10 of the 12 individual dent corn samples grown in 1955 showed low but definite levels of BHC in the grain, the exception being Pioneer single-cross GN2 $\times$LF5 grown on soil treated with BHC in 1955.

In general sweet corn seed contained small amounts of insecticide following the soil treatments employed in these experiments. The two individual samples of Iochief grown in 1954 averaged 0.71 p.p.m., while the same variety grown on the same plots in 1955 averaged 0.41 p.p.m. Adjacent plantings of Tendermost in 1955 averaged .04 p.p.m. Seven out of the eight sweet corn samples from the plots treated just before planting in 1955 showed insecticide present, the range being .02 to .37 p.p.m.

Popcorn was included in the 1955 plantings, and the harvested seed was very low in insecticide content. When the duplicate samples were averaged they ranged from zero to 0.13 p.p.m., and at least one replicate of each of the five pairs showed no insecticide present.

Other crops investigated were sunflowers, grain sorghum and garden beans. The samples of these crops grown on the plots treated with 50 pounds per acre of lindane in 1952 consistently gave positive results during the four-year period. However the single samples of this group of crops harvested from the plots treated with 10 pounds per acre of insecticide just before planting in 1955 showed seven zero and only three positive values.

Two samples of Great Northern beans grown from seed containing 0.13 p.p.m. of lindane planted on untreated soil showed questionable traces of this insecticide in 1954.

In view of the excessive dosages used on these plots, and the minute amounts of insecticide recovered from the various plant samples, there seems little possibility that translocation will cause residues anywhere near the official tolerances of 10 p.p.m. for lindane and 5 p.p.m. for BHC deemed safe to man on fruits and vegetables (Federal Register, Vol. 20, No. 49, page 1494, March 11, 1955).

A preliminary test in which confused flour beetle adults were fed on crushed sweet and dent corn kernels from the various 1955 samples showed no apparent adverse effects of the translocated BHC in the grain on the test insects under the conditions of this experiment. However the test insects did much better on dent corn than they did on sweet corn.

Official germination tests of corn and soybeans grown on soil treated with 50 pounds per acre of lindane shortly before planting in 1952 gave results in good agreement with those from untreated control samples of both crops.

Popping expansion tests on the popcorn samples grown in 1955 did not show any differences that can logically be attributed to soil treatments with lindane or BHC applied before the corn was planted.

Taste and smell tests on 14 popcorn samples revealed no indications of abnormal tastes or odors resulting from excessive applications of insecticide to the soil in which the plants were grown.

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Tobacco Hornworm Control Experiments in North Carolina, 1953–1955[1]

R. L. RABB[2] and F. E. GUTHRIE[2]

The tobacco hornworm, *Protoparce sexta* (Johan.), is the major insect pest of flue-cured tobacco. The most important literature pertaining to its insecticidal control was reviewed by Allen *et al.* (1953), who also reported results of their control experiments conducted during 1950 through 1952. They found TDE, endrin, Dilan, and Metacide to be promising for hornworm control. Endrin, applied as emulsion sprays at rates of 0.3 and 0.6 pounds active ingredient per acre killed a greater percentage of hornworms and was faster in lethality than other materials tested. They also found that a 1% endrin dust was very toxic to this pest in a preliminary test.

Endrin was recommended for hornworm control in 1954 as a spray but not as a dust. This situation was caused by lack of information on the performance of dusts and to the fact that some difficulty had been encountered in manufacturing stable dust formulations.

Toxaphene, although not recommended on tobacco because of its deleterious effects on flavor and aroma, is nevertheless being used in considerable quantities for tobacco hornworm control. Its performance as compared to the currently recommended materials has been the subject of many inquiries from tobacco growers. Insecticidal tests conducted in North Carolina against the tobacco hornworm in 1953, 1954, and 1955 are herein reported.

PROCEDURE.—Plots consisted of 1 or 2 rows of tobacco, separated from adjacent plots by 3 or more guard rows. Pre-treatment and post-treatment counts of hornworm larvae were made on a given number of infested plants, marked with stakes. In order to have approximately the same number of larvae per plot, larvae were carefully moved from unmarked to marked plants when necessary. Parasitized and abnormal larvae were discarded.

In 1953, two experiments were conducted to compare the performance of endrin, Strobane and TDE sprays. In experiment 1, sprays were applied with a Boyette sprayer similar to that described by Allen *et al.* (1953), except that it was self-propelled. A hand-operated, 3-gallon, compression-type sprayer was used in experiment 2. Spray material was applied at approximately 35 to 40 gallons per acre.

In 1954, four small plot experiments and three large-scale field trials were conducted in an effort to evaluate the effectiveness of several endrin dust formulations as compared to the currently recommended 10% TDE dust. Dusts were applied with rotary hand dusters at approximately 20 to 30 pounds per acre, depending primarily on the size of the plants.

In 1955, one small-plot experiment was conducted to compare the effectiveness of toxaphene, endrin, TDE and cryolite. Dusts were applied with rotary hand dusters and sprays with a self-propelled, high-clearance sprayer, operating at 60 p.s.i. This sprayer delivered approximately 35 gallons per acre through seven nozzles per row, except as noted under "TDE Tip Spray" in table 3.

RESULTS AND DISCUSSION.—Results of the 1953 experiments are given in table 1. TDE, endrin, and Strobane sprays gave excellent hornworm control. Endrin at 0.2 and 0.4 pound per acre was the most rapidly acting toxicant.

[1] Accepted for publication May 4, 1956.

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Table 1.—Effectiveness and speed of action of several emulsion sprays for tobacco hornworm control.[a] 1953.

Emulsion Concentrate	Pounds Active Ingredient per Acre	Number Larvae Treated[b]	Per Cent Reduction in Living Larvae After (Hours)				Per Cent of Larvae Dead or Moribund After (Hours)				Per Cent Larvae Missing After 72 Hours
			6	24	48	72	6	24	48	72	
Experiment 1											
25% TDE	1.0	221	8.6	71.0	92.8	98.6	4.1	65.2	84.6	89.1	9.5
18½% endrin	0.2	224	51.3	78.1	96.4	97.8	45.5	71.9	89.7	91.1	6.7
	0.4	217	73.3	92.2	98.6	99.1	68.5	84.8	92.6	93.1	6.0
42% Strobane	2.0	227	18.5	61.7	86.8	93.8	13.2	54.2	72.2	77.5	16.3
Experiment 2											
25% TDE	1.0	79	13.9	84.8	95.0	98.7	3.8	70.9	74.7	77.2	21.5
18½% endrin	0.2	87	70.1	88.5	100.0	100.0	59.7	78.1	89.7	89.7	10.3
	0.4	81	81.5	87.7	98.8	100.0	72.8	78.8	80.3	81.5	18.5
42% Strobane	2.0	89	20.2	68.5	93.3	99.0	15.7	57.3	84.3	90.0	9.0

[a] Data based on larval counts on 20 plants per treatment in experiment 1 and 10 plants per treatment in experiment 2.
[b] Hornworms were predominantly 4th and 5th instars, though a few large 3rd instars were included.

Data from the 1954 tests are given in table 2. All treatments gave satisfactory control after 48 hours. On the basis of the 9-hour counts in experiments 1, 2 and 3, both of the 2% endrin formulations were somewhat slower in action than the ¾% and 1½% endrin dusts. This discrepancy was found to be due to the decomposition of endrin in the 2% dusts, which were formulated with active diluents without the inclusion of a deactivator. This decomposition amounted to 42% in formula A and 50% in formula B. Chemical analysis also showed that the ¾% and 1½% dusts, which were formulated with diluents deactivated with hexamethylenetetramine, were somewhat higher in endrin content than their stated strength.

Table 2.—Field tests with endrin and TDE dusts against the tobacco hornworm in North Carolina during 1954.

Treatment[a]	Number of Larvae Treated[b]	Per Cent Reduction in Living Larvae After Treatment		
		9 Hours[c]	24 Hours	48 Hours
Experiment 1 and 3[d]				
¾% endrin	300	78	93	99
1½% endrin	308	75	86	99
2% endrin A	298	69	91	99
2% endrin B	301	59	84	98
10% TDE	295	55	98	100
Check	303	15	30	48
Experiment 2				
¾% endrin	96	50	77	95
1½% endrin	96	52	82	98
2% endrin A	95	37	59	87
10% TDE	90	37	92	100
Check	98	6	16	31
Experiment 4				
¾% endrin	91	19	86	100
2% endrin A	89	36	93	100
2% endrin B	89	25	85	98
Check	90	8	9	19

[a] Three replications per treatment. Hornworm counts were made on 5 plants per plot in experiment 1, 25 plants in experiment 2, 20 plants in experiment 3, and 10 plants in experiment 4.
[b] Hornworms in experiments 1, 2, and 3 were 3rd, 4th, and early 5th instars. Only early 5th instars were used in experiment 4.
[c] This count was made at 6 hours in experiment 1 and 14 hours in experiment 4.
[d] These two experiments were identical; data from them were combined.

Table 3.—Effectiveness of insecticides against the tobacco hornworm. 1955.

Treatment[a]	Pounds Actual per Acre	Number of Larvae Treated[b]	Per Cent Reduction in Living Larvae After Treatment		
			24 Hrs.	48 Hrs.	96 Hrs.
TDE, 25% E.C., Tip spray[c]	0.13	135	45	66	76
TDE, 25% E.C., Reg. spray[d]	0.88	123	76	89	—
TDE, 10% dust	2.40	130	86	91	—
Endrin, 19½% E.C., Reg. spray	0.30	132	93	99	—
Endrin, 1½% dust	0.33	122	88	96	—
Toxaphene, 60% E.C., Reg. spray	1.60	130	85	93	—
Cryolite, 70% dust	14.70	130	35	63	88
Check	—	137	11	18	32
L.S.D. at 5% level			7.9	8.1	
at 1% level			10.9	11.3	

[a] Three replicates. Hornworm counts made on 10 plants per replicate.
[b] 41% 5th instar and 59% 3rd and 4th instar larvae.
[c] Applied to the top of the plants through one nozzle per row.
[d] Applied for complete coverage of foliage through seven nozzles per row.

Satisfactory endrin dusts are now being formulated through the use of inert or deactivated diluents. The 10% TDE, although somewhat slower in action initially, gave as good or slightly better control than the endrin dusts in these tests. In the three large-scale field trials, ¾% and 1½% endrin dusts gave excellent hornworm control.

Results of the 1955 experiments are shown in table 3. Endrin spray was the best treatment and TDE tip spray and cryolite dust were the poorest treatments on the basis of the 24-hour count. At the 48-hour count, TDE tip spray and cryolite dust were again markedly poorer than other treatments. Of the other treatments, only TDE regular spray was significantly poorer than endrin spray, the best treatment. Although the 96-hour count indicated that the TDE tip spray and the cyrolite dust had brought about fair to good control, these treatments were too slow in action to be considered satisfactory. In fairness to tip sprays, it should be stated that they would likely be more effective and probably satisfactory if applied as suggested by Lawson,[3] i.e., at the time the first fourth-instar larvae appear, when practically all larvae are on the top third of

[3] The idea of controlling hornworms through properly timed "tip" sprays originated with F. R. Lawson, U. S. Dept. Agric., Agric. Res. Serv. Ent. Res. Branch, Oxford, N.C.

the plants. Such an application would also be directed against much smaller larvae than those utilized in this experiment.

SUMMARY.—In tests conducted against *Protoparce sexta* (Johan.) in North Carolina during 1953–1955, endrin sprays were found to be faster in action than TDE, Strobane, or toxaphene sprays but all materials were equal in effectiveness 48 hours after treatment. Properly formulated endrin dusts were found as satisfactory as sprays. Cryolite dust was unsatisfactory when compared with the newer organic insecticides. TDE sprays directed through one nozzle per row to the top of plants failed to satisfactorily control large larvae.

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The Fate of Radiophosphorus Ingested by House Flies and German Cockroaches[1]

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Attempts have recently been made in this laboratory to impair development in insects by feeding them various substances (Mitlin *et al.* 1954, Mitlin & Konecky 1955, Konecky & Mitlin 1955). Hoffman & Lindquist (1951) using house flies, Grosch & Sullivan (1953) using the parasitic wasp *Habrobracon juglandis* (Ashmead), and King (1954) using *Drosophila melanogaster* (Meig.) found that the fertility of the insects was affected adversely by the ingestion of radiophosphorus (P^{32}). Babers & Roan (1954) studied the distribution of radiophosphorus in house flies reared on P^{32}-containing medium, but used rather low intensities of radioactivity. More recent experiments with radiophosphorus on house flies and German cockroaches are described herein.

TESTS WITH HOUSE FLIES.—Susceptible house flies (*Musca domestica* L.) from the regular laboratory colony reared according to the Peet-Grady method (Anon. 1953) and normal German cockroaches (*Blattella germanica* (L.)) fed on dog pellets were used. The radioactive phosphorus[3] was in the form of $KH_2P^{32}O_4$ solution and had an activity of 0.459 mc./ml. at the start of the experiments.

To 5 grams of dried skimmed milk was added 5 ml. (2.295 mc.) of the P^{32} solution. After thorough mixing, it was made accessible to newly emerged house flies that had not fed. Water was given separately.

After 3 hours 10 flies of each sex were removed, and their individual radioactivities were determined after the insect had been placed in a planchet under carbon dioxide anesthesia and the planchet covered with thin aluminum foil (4.31 mg./cm.^{2}).

Immediately after their removal from the radioactive food, the male flies had an activity corresponding to that of 1.9 mg. of the dried milk and each female that of 1.8 mg. of milk.

After their activity had been determined, individual insects were placed in pint fruit jars and allowed to feed normally on cotton wetted with skimmed milk. The radioactivity of each insect was then observed for 20 days. After corrections for radio decay, the results were plotted as per cent of initial activity versus time. Composite curves for 10 male and 10 female flies are shown in figure 1. The feces from these flies and the cotton wet with milk upon which they fed were very radioactive for the first 8 days.

After being kept in separate containers for 11 days, the insects were put into one cage. Copulation started within 5 minutes. Two days later 114 eggs were deposited that had an activity of 1313 c/m. Only 13 hatched, and from these 13 larvae, 2 adult flies, both males were obtained.

Other newly emerged house flies were allowed to feed for 3 hours on the radioactive dried milk described above and then put on the regular milk diet. When 5 days old, eggs were collected in the usual manner. When placed in an aluminum planchet, 0.1 ml. of eggs (about 500) had an activity of 9353 c/m. when counted with a 1.4 mg./cm.^{2} window G.M. tube. Of these 55% hatched normally. From these eggs 31 adults were obtained, 11 males and 20 females.

Still another group of flies was removed from the cage containing the radioactive food after feeding for 3 days and placed on normal milk. When they were 5 days old (2 days on normal milk) eggs were collected. Only 18.5% of these eggs were viable, and 0.1 ml. had a radioactivity of 12500 c/m. Another group that was fed on P^{32}-containing milk for 6 days did not oviposit. However, when these flies were placed on normal milk for 2 days, a few eggs were collected, and these were 11.2% viable. About 100 eggs had an activity of 12274 c/m. Figure 3 shows the ovaries of house flies that had fed on food containing P^{32} for different periods. The medium on which the resulting larvae were placed developed mold, and the larvae died over a weekend. No conclusions could be drawn, but the larvae apparently developed normally for several days.

In an experiment to determine the effect of rearing larvae on P^{32}-containing medium, 0.1 ml. of eggs from the regular laboratory colony was added to 300 grams (dry weight) of regular Chemical Specialties Manufacturers

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[2] Resigned March 30, 1956. Present address: Chemicals and Plastics Division, Quartermaster Laboratories, U. S. Army, Natick, Mass.

[3] Supplied by M. E. Jefferson under the authority of the United States Atomic Energy Commission.

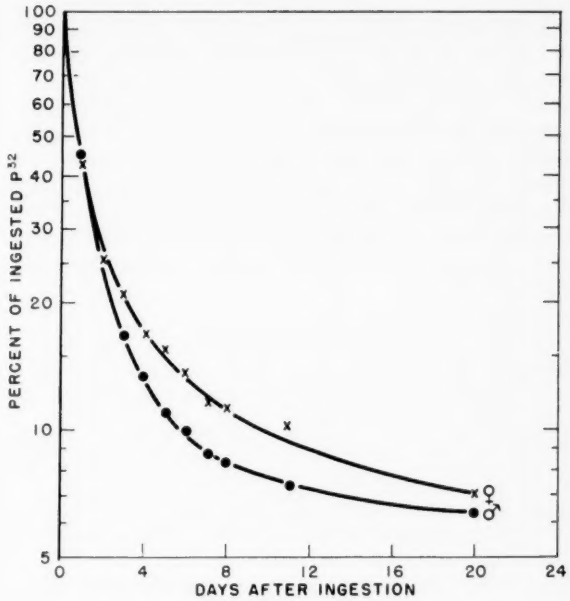

FIG. 1.—Elimination of P^{32} by house flies.

Association medium containing varying quantities of P^{32}. The results are shown in table 1. Flies from these larvae oviposited normally and had normally fertile eggs.

In common with most insects, the house fly probably absorbs only a small percentage of the food it ingests. In order to learn how much time is required for ingested food to pass through the insect, newly emerged unfed adults, both male and female, were fastened at the dorsal thorax to small wooden applicators with cellulose cement. The two rear legs of each insect were then clipped, and the applicator was clamped so that the abdomen projected onto a counting disc. Cotton moistened with milk containg P^{32} was placed so that the insect could feed. After feeding started, the time before the first defecation varied between $1\frac{3}{4}$ and $2\frac{1}{4}$ hours. In each case the feces were highly radioactive.

TESTS WITH COCKROACHES.—Five adult German roaches of each sex were allowed to feed for 24 hours on a meat-base dog pellet weighing 2.8 grams that had been moistened with a $KH_2P^{32}O_4$ solution containg 0.61 mc of P^{32}. The insects were placed on normal food, and the radioactivity of individual roaches was determined periodically. The results are shown in figure 3.

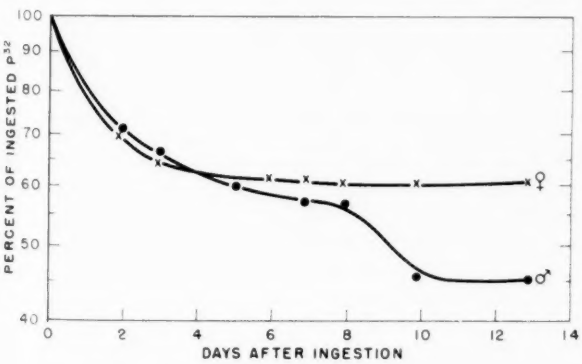

FIG. 2.—Elimination of P^{32} by German cockroaches.

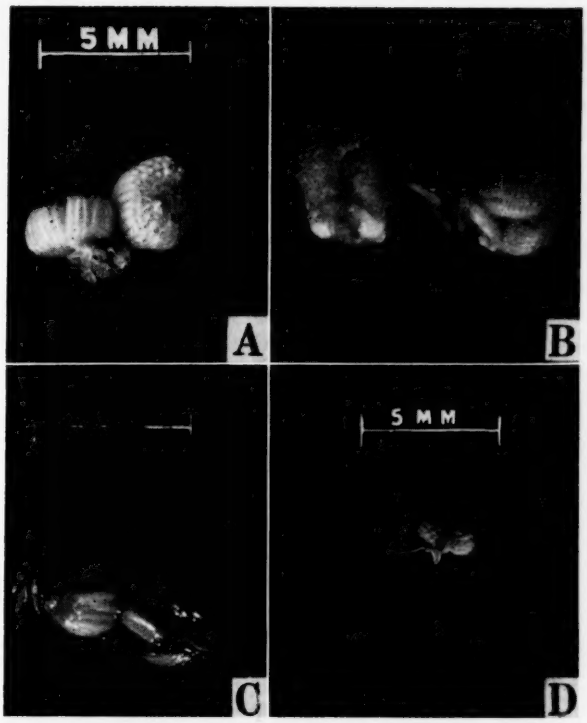

FIG. 3.—Effect of feeding P^{32} on the ovaries of house flies: Normal (A), returned to normal diet after feeding 3 hours (B), 3 days (C), and 6 days (D).

During the course of the experiment four of the female cockroaches developed oöthecae. They were very flaccid and contained no eggs. However, the capsules were highly radioactive, yielding counts of several hundred per minute.

From their radioactivity at the time they were changed from radioactive to regular food, it was determined that each male cockroach contained the equivalent of 0.37 mg. of dog biscuit and each female 0.48 mg.

DISCUSSION.—Fifty per cent of the ingested radiophosphorus present in adult house flies feeding for 3 hours on the marked food had disappeared in about 19 hours. The rapid drop in activity continued until about the fourth day, when it began to level off. This is in contrast with the

Table 1.—Results of rearing house fly larvae on medium containing P^{32}.

ACTIVITY OF MEDIUM (μc./GRAM)	SPECIFIC ACTIVITY OF "PUPA"[a] (c/m)	PER CENT EMERGENCE OF ADULTS	SPECIFIC ACTIVITY OF PUPARIUM (c/m)
15.3	16900	100	1395
7.7	11900	82	953
4.0	6580	100	627
2.0	3650	100	431
1.0	1880	83	194
0.5	1165	100	96

[a] "Pupa" is here used to designate the puparium plus all contents. It is recognized that this use of the term is incorrect, but a survey of opinion of competent entomologists indicates that there is no proper term with this meaning.

several-day biological half-life observed by Babers & Roan (1954) in adult flies that had developed from larvae reared continuously on P^{32}-containing medium, and also is in contrast with the experience of Grosch & Sullivan (1953) with *Habrobracon*. In that insect the biological half-life of P^{32} ingested by adults was almost 5 days. The difference may well be due to differences in feeding habits. The house fly regurgitates frequently while feeding, and a large amount of the P^{32} ingested was excreted in this way.

The small immature ovaries noted in the flies fed several days on the P^{32}-containing medium were similar in appearance to those described by Mitlin *et al.* (1956) as occurring after the feeding of various mitotic poisons. The effect of the true mitotic poisons was not reversible. Ovaries of adult flies maintained on sugar water were also immature, but after the insects were placed on an adequate diet egg production and viability were soon normal. The ovaries of flies maintained on the P^{32}-containing diet were also immature, but they did not completely recover when a normal diet was furnished. Both the production and viability of the eggs were greatly reduced.

The radiophosphorus in the female cockroaches did not fall to 50% of the starting level in the 14-day observation period. The level in the male insects followed that in the females rather closely until the eighth day, when a rapid fall occurred. The curves in figure 2 are a composite of those for five individuals, but the sudden drop was noted in all. No explanation is offered.

SUMMARY.—House flies (*Musca domestica* L.) and German cockroaches (*Blattella germanica* (L.)) were fed radiophosphorus to study its effect on their fertility. In house flies the biological half-life of the ingested P^{32} was about 0.8 day, whereas in female cockroaches the radioactivity did not fall to 50% in the 14-day observation period. In male cockroaches this point was reached about the ninth day. House flies fed P^{32}-labeled food continuously for 6 days did not oviposit, but 2 days after they had been returned to a normal diet a few eggs of low viability were deposited. Flies kept on the P^{32} for shorter periods showed inhibited oviposition and fertility. Their ovaries were immature, but they recovered partially after a normal diet was given. Larvae reared on P^{32} medium (15.3 μc./gram) developed into normal adults. Some German cockroaches fed P^{32} developed oöthecae, but they were flaccid and contained no eggs.

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Experiments on Earworm Control on Sweet Corn[1]

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Great progress has been made within the past few years on control of the corn earworm, *Heliothis zea* (Boddie), on sweet corn. It is established that DDT is probably the best insecticide for this purpose (Blanchard *et al.* 1955) and is currently recommended in most areas (Anderson *et al.* 1949, Blanchard *et al.* 1951, Ditman 1950, Eden 1951, Rutschy 1951, Wene & Blanchard 1949, Hawkins 1955, Pond 1955, Wilson 1955). Timing of spray applications, nozzle types, amount of DDT, and use of mineral oil influence the degree of earworm control obtained on sweet corn. Results of research on timing of sprays have been reported by several investigators under various conditions (Thames 1952, Ditman *et al.* 1955, Eden 1952). In general, a 3-day interval between sprays has been recommended. Work on different types of nozzles has been reported by Howe & Davis (1954), Wilson (1952), Eden (1952) and Black *et al.* (1954). In general, four flat fan nozzles per row have been recommended.

Experiments involving some of these factors have been conducted in Alabama for several years. Some of the experiments conducted during the period 1953–55 are reported in this paper.

MATERIALS AND PROCEDURE.—The variety of sweet corn involved in the experiments reported in this paper was Aristogold Bantam Evergreen. Each plot consisted of four rows of sweet corn from 70 to 100 feet long. The treatments in all experiments were replicated four times in randomized blocks. All sprays were applied with a high-clearance, self propelled power sprayer. Unless otherwise indicated in the individual experiments, the machine was equipped with four flat fan nozzles (650067) per row. Sprays were usually applied at 100 p.s.i. and in a volume of 20 gallons per acre. All spray formulations were emulsions. Results in all experiments were determined by examining 100 consecutive ears on the two inside rows of the plots when the corn was ready to harvest. All experiments

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reported herein were conducted near Fairhope, Alabama at the Gulf Coast Substation of the Agricultural Experiment Station, Alabama Polytechnic Institute.

In 1953, an experiment was conducted to determine the effects of varying rates of DDT, with and without 1.75 gallons of mineral oil per acre, on control of the earworm. The rates of DDT were 1.0, 1.5, 2.0, and 2.5 pounds per acre per application. Five sprays were applied at 3-day intervals, beginning on May 9 when 5% of the stalks were in silk. The corn was harvested on May 29. This experiment was part of a national cooperative experiment conducted that year.

In 1954, an experiment was conducted that was similar to the 1953 experiment but somewhat expanded. Rates of DDT of 1.0, 1.5, 2.0, and 2.5 pounds per acre per application were applied with and without 1.75 gallons of mineral oil per acre. DDT rates of 3 and 4 pounds per acre per application were used without mineral oil. Seven sprays were applied at 3-day intervals, beginning on May 14, at which time less than 1% of the stalks were in silk. The corn was harvested on June 7. Part of this experiment was included in a national cooperative corn earworm control experiment in 1954.

In 1955, two experiments were conducted. Experiment I was designed to determine the effects of timing and number of applications of DDT-mineral oil emulsion sprays on earworm control. All treatments received 2 pounds of DDT and 1 gallon of mineral oil (Carnation White) per acre per application. Variables in the experiment were time of beginning the spraying program, intervals between applications, and the number of sprays applied. The spraying program was begun on May 24 and continued to June 6 for certain treatments. The entire spraying program was delayed 1 day on May 29 because of a heavy rain. The silking percentages by dates are given in table 1. The corn was harvested on June 10. This experiment was part of a national cooperative experiment on corn earworm control in 1955.

Experiment II conducted in 1955 was designed to determine the effects of various types of spray nozzles, intervals between spray applications, and rates of DDT on earworm control. Nozzles used were flat fan (Spraying Systems Tee-jet 650067), hollow cone (Spraying Systems Tee-jet T-2), and a high-gallonage (John Bean) agricultural nozzle equipped with a No. 3 disc. The flat fan and hollow cone nozzles were used at 150 p.s.i., and the high-gallonage nozzle was used at 80 p.s.i. The first sprays were applied to all plots on May 23, at which time 2.5% of the stalks were showing silks (table 1). The spraying program was continued until June 8; the corn was harvested June 10.

RESULTS.—Results of the 1953 experiment are presented in table 2. All spray treatments resulted in significantly more worm-free ears than no treatment. Where no mineral oil was used, the worm control increased as the rate of DDT was increased. Although 1.5, 2.0, and 2.5 pounds of DDT gave significantly better control than 1.0 pound, the differences between 1.5 and 2.0 pounds or 2.0 and 2.5 pounds were not significant. Where 1.75 gallons of mineral oil was used per acre per application, 1.5 pounds of DDT was significantly better than 1.0 pound; however, 2.0 pounds was not significantly better than 1.5 pounds per acre per application. The use of the mineral oil significantly increased the earworm control when used with 1.0 and 1.5 pounds of DDT per acre. The difference between results obtained with oil and no oil at 2.0 pounds of DDT approach significance. Two and one-half pounds of DDT per acre with no mineral oil resulted in essentially the same earworm control as 2.0 pounds of DDT with the 1.75 gallons of mineral oil. In all cases where mineral oil was included in the sprays, foliage injury resulted.

Results of the 1954 experiment are presented in table 3. The earworm infestation was severe, and there were no worm-free ears on the unsprayed corn. All spray treatments were highly effective in reducing earworm damage. Where no mineral oil was used, the percentages of worm-free ears increased significantly with each 0.5-pound increment of DDT up to 2.5 pounds per acre per application. Increasing the rate to 3 or 4 pounds per acre without

Table 1.—Silking rate of sweet corn used in experiments on control of corn earworm. Gulf Coast Substation, 1955.

DATE	PER CENT OF STALKS SHOWING SILKS
May 23, 4:00 P.M.	2.5
May 24, 9:00 A.M.	9.8
5:00 P.M.	17.7
May 25, 8:00 A.M.	31.7
May 26, 10:00 A.M.	57.0
May 30, 8:00 A.M.	95.0

Table 2.—Worm-free ears of sweet corn after spraying with varying rates of DDT with and without mineral oil for earworm control. Gulf Coast Substation, 1953.

DOSAGE PER ACRE	WORM-FREE EARS	
	Per Cent	*Angle*
Untreated	13.5	21.25
DDT, 1 lb.; mineral oil, 1.75 gal.	82.0	65.34
DDT, 1 lb.	51.8	46.04
DDT, 1.5 lb.; mineral oil, 1.75 gal.	90.9	72.64
DDT, 1.5 lb.	73.7	59.38
DDT, 2.0 lb.; mineral oil, 1.75 gal.	88.0	69.99
DDT, 2.0 lb.	80.3	63.65
DDT, 2.5 lb.	85.3	67.65
L.S.D. at 5% level		6.96
at 1% level		9.48

Table 3.—Worm-free ears of sweet corn following various sprays containing DDT for control of corn earworm. Gulf Coast Substation, 1954.

DOSAGE PER ACRE	WORM-FREE EARS	
	Per Cent	*Angle*
Untreated	0	0.00
DDT, 1.0 lb.; mineral oil, 1.75 gal.	58.8	50.09
DDT, 1.0 lb.	17.5	24.46
DDT, 1.5 lb.; mineral oil, 1.75 gal.	74.5	60.01
DDT, 1.5 lb.	42.8	40.72
DDT, 2.0 lb.; mineral oil, 1.75 gal.	81.0	64.26
DDT, 2.0 lb.	53.3	46.86
DDT, 2.5 lb.; mineral oil, 1.75 gal.	86.8	68.88
DDT, 2.5 lb.	64.5	53.50
DDT, 3.0 lb.	54.0	47.29
DDT, 4.0 lb.	67.5	55.28
L.S.D. at 5% level		6.04

mineral oil did not give corresponding increases of worm-free ears. At the 1.0-pound rate of DDT, the use of mineral oil increased the percentage of worm-free ears from 17.5 to 58.8. At the 2.5-pound rate of DDT, the mineral oil resulted in an increase of worm-free ears from 64.5 to 86.8%. Thus, the use of mineral oil was significantly effective in increasing the earworm control. Moderate foliage injury occurred on the plots that received mineral oil.

Results of the 1955 experiment on timing and number of applications of DDT-mineral oil sprays are presented in table 4. The first sprays were applied the first day after the first silks appeared, when 9.8% of the stalks were in silk. Those started on the second day after the first silks appeared were applied when 31.7% of the stalks were in silk. All spray treatments were significantly effective in reducing the earworm infestation. The most effective treatments were 5 and 6 applications at 2-day intervals beginning the first day after the first silks appeared. There was no significant difference between the controls obtained with 4 and 5 applications at 3-day intervals beginning the first day after the first silks appeared. Furthermore, there were no significant differences between the controls obtained with 4 and 5 applications at 3-day intervals beginning the first day after the first silks appeared and those obtained by 6 applications at 2-day intervals or 4 applications at 3-day intervals begun the second day after the first silks appeared. Thus in this experiment sprays applied at 2-day intervals resulted in better earworm control than those applied at 3-day intervals, and sprays begun the first day after the first silks appeared gave better earworm control than those begun the second day after the first silks appeared.

Results of the 1955 experiment on nozzles, intervals, and rates of DDT are presented in table 5. There was an average of 8.5% of the ears worm-free in the untreated checks. The best control obtained was 93.5% worm-free ears which resulted from 16 daily applications of 2 pounds of DDT applied with flat fan nozzles. The percentage dropped to 75.5 at 2-day intervals and 67.3 at 3-day intervals, these differences being significant. Likewise, this difference in control due to interval also occurred when hollow cone nozzles were used. When 2.0 pounds of DDT was applied at 3-day intervals, the percentages of worm-free ears were 67.3, 41.3, and 61.3 for flat fan, hollow cone, and agricultural nozzles, respectively. The flat fan and agricultural nozzles were significantly better than the hollow cones. When flat fan nozzles and 3-day intervals were employed, the worm-free ears resulting from 2.0, 2.5, and 3.0 pounds of DDT per acre were 67.3, 72.8, and 73.3%, respectively, and the differences were not significant. Hence, it is evident from these data that the interval between spray applications was highly significant in earworm control, that flat fan and agricultural type nozzles were better than hollow cones, and that 3 pounds of DDT was little better than 2 pounds per acre per application at 3-day intervals.

Table 4.—Worm-free ears of sweet corn following application of various DDT spray treatments for earworm control. Gulf Coast Substation, 1955.

Number and Timing of Treatments	Worm-Free Ears	
	Per Cent	*Angle*
Untreated	11.0	19.14
Begin first day after first silk:		
6 applications at 2-day intervals	90.5	72.40
5 applications at 2-day intervals	91.8	73.36
5 applications at 3-day intervals	81.0	64.28
4 applications at 3-day intervals	77.8	62.02
Begin second day after first silk:		
6 applications at 2-day intervals	76.8	61.39
5 applications at 3-day intervals	74.5	59.71
L.S.D. at 5% level		5.36

Table 5.—Control of corn earworm on sweet corn with various types of nozzles, spray intervals, and rates of DDT. Gulf Coast Substation, 1955.

Spray Interval	Pounds of DDT per Acre per Application	Total No. of Sprays	Worm-Free Ears	
			Per Cent	*Angle*
Untreated		0	8.5	14.70
Four flat fan nozzles per row				
1-day interval	2.0	16	93.5	77.70
2-day intervals	2.0	8	75.5	60.81
3-day intervals	2.0	6	67.3	55.16
3-day intervals	2.5	6	72.8	58.79
3-day intervals	3.0	6	73.3	58.90
Four hollow cone nozzles per row				
2-day intervals	2.0	8	63.8	53.03
3-day intervals	2.0	6	41.3	39.85
Two high-gallonage agricultural nozzles per row				
3-day intervals	2.0	6	61.3	51.53
L.S.D. at 5% level				8.40

SUMMARY.—Experiments were conducted in Alabama during 1953, 1954, and 1955 on control of the corn earworm, *Heliothis zea* (Boddie), on sweet corn. In the experiments in 1953 and 1954 where sprays were applied at 3-day intervals, the earworm control increased as the rate of DDT per acre per application was increased from 1.0 to 2.5 pounds. Higher rates of DDT did not give corresponding increases in control. The use of 1.75 gallons of mineral oil per acre per application resulted in significant increases in earworm control over no oil; however, when mineral oil was included in the sprays, foliage injury resulted.

In 1955, an experiment was conducted to determine the effects of timing and number of applications of DDT-mineral oil emulsion sprays on earworm control when 2 pounds of DDT and 1 gallon of mineral oil were used per acre per application. Sprays applied at 2-day intervals resulted in better earworm control than those applied at 3-day intervals, and sprays begun the first day after the first silks appeared gave better earworm control than those begun the second day after the first silks appeared.

A second experiment was conducted in 1955 to determine the effects of various types of spray nozzles, intervals between applications, and rates of DDT on earworm control. One-day interval between sprays resulted in the best earworm control and was followed by 2- and 3-day intervals in that order. There was no difference in controls obtained with flat fan and agricultural-type nozzles, but both were better than hollow cones. Three pounds of DDT per acre per application was little better than 2 pounds applied at 3-day intervals.

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Artificial Pollination of Apples with Bee-Collected Pollen[1]

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Artificial pollination of apples has been adopted as a horticultural practice under certain conditions. This practice is used as a supplement to natural insect pollination when self-barren varieties such as Delicious and Winesap are planted in solid blocks and when insect pollinator activity is inadequate due to inclement weather conditions. It has also become increasingly difficult to obtain honey bees for pollination service because of losses to the beekeepers caused by bee poisoning and poor honey yields.

At present, commercial-scale use of this technique is by means of hand-collected pollen applied with a brush (Snyder 1947). Devices for coating honey bees with hand-collected pollen to obtain the required pollinating effects have been unsuccessful (Webster *et al.* 1949, Bullock 1947, Griggs *et al.* 1952). Since hand-collected pollen retains its viability in storage longer than bee-collected pollen, it has been the preferred material for orchard use (Singh & Boynton 1949, Vansell & Griggs 1952). However, large amounts of pollen material can be obtained more cheaply by means of pollen traps placed on beehives than by hand collecting. Therefore, this study was undertaken in an effort to develop the use of bee-collected pollen for hand pollination (Johansen 1955).

METHODS AND MATERIALS.—Bee-collected pollen was obtained by means of pollen traps similar to the type portrayed by Schaefer & Farrar (1946). Hives with these attachments were placed in apple orchards at the peaks of blooming periods. Using the techniques of Griggs *et al.* (1950), pollen samples were removed from the traps at intervals of 1 hour or less, and placed in 2-dram vials in an insulated box containing dry ice. Later these samples were stored at 0° F. for use in various tests.

Hand-collected pollen was obtained by scraping apple blossoms in the "balloon" stage across an 8-mesh per inch hardware-cloth-capped quart jar. The anthers collected were cured at 75° F. for 72 hours and stored at 0° F.

Germination tests of the stored pollen were conducted periodically, using the techniques of Griggs *et al.* (1953). Pellets were mixed with a small amount of 15% sugar syrup and plated on freshly-prepared 15% sugar plus 2% agar medium. The petri plates were held at 75% F. and checked after 24 hours.

Pollination tests were conducted with sleeve cages on individual apple branches in 1954 and by means of a large tree cage (fig. 1) in 1955. Samples were applied by hand with a No. 4 hog-bristle brush at intervals throughout the blooming periods. A standard lycopodium spore material[3] was used as a diluent in formulating the pollen dusts in 1955. Powdered skim milk was used in 1954.

FIELD OBSERVATIONS.—The first attempts to obtain bee-collected pollen at Wawawai in 1954 failed. Only a limited amount of apple blooms were present in the orchard and the honey bees collected almost all of their pollen from balsam-root (*Balsamorhiza sagittata*) on a distant hillside.

Later in 1954 and in 1955, hives were placed in a large orchard of Rome Beauty apples at Dayton in the peak of bloom. These attempts were successful, the bees collecting pure apple pollen until the morning of the third day, when mixed pollen loads containing mustard (*Brassica campestris*) or dandelion (*Taraxacum officinale*) in addition to apple began to appear.

Table 1 indicates the percentages of pollen collectors observed and the average number of pollen pellets collected in various time intervals during these investigations. These observations are similar to those of other investigators, showing that the greatest amount of pollen

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[3] S. B. Penick & Co., New York, N. Y.

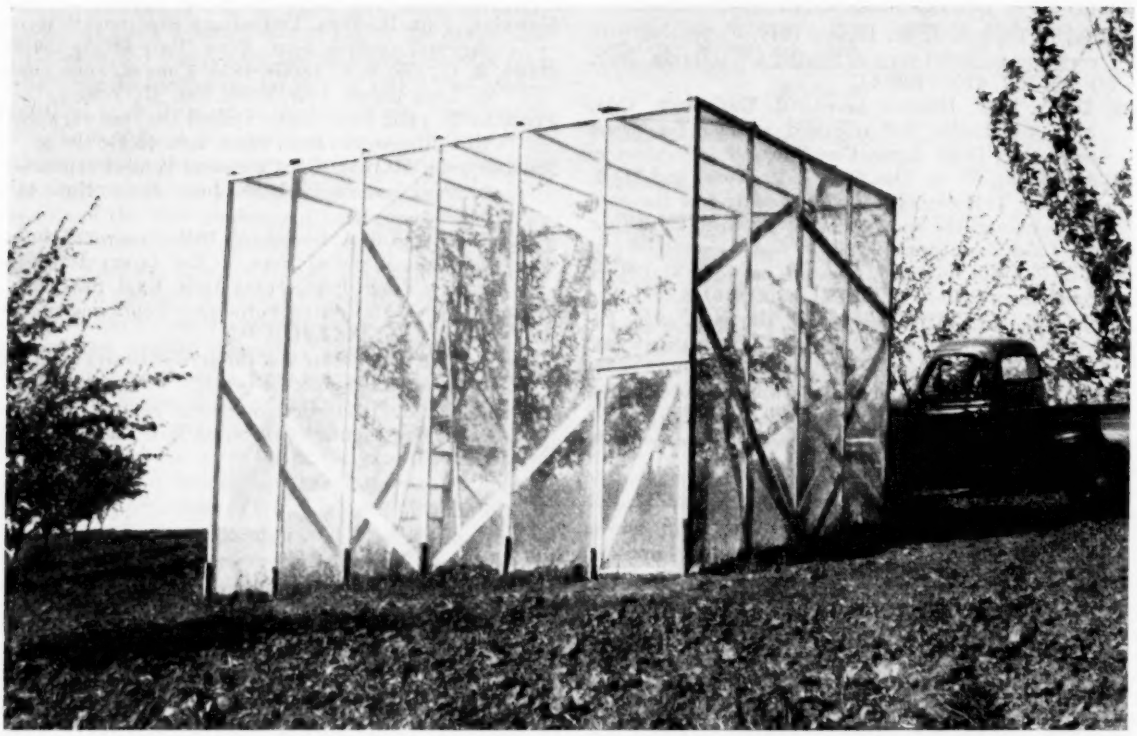

FIG. 1.—Tree cage used in pollination studies (dimensions: 14 feet at highest point, 18 feet long, and 12 feet wide).

is brought into the hives during the morning and the greatest proportion of pollen collectors are active at that time.

RESULTS OF GERMINATION TESTS.—Pollen samples were held at 0° F. under various conditions and treatments. At intervals, portions of these samples were removed from storage and tested for viability. The initial viability of bee-collected pollen was 78% and of hand-collected pollen, 89%. At room temperature (75° F.), the viability of bee-collected pollen was reduced to 11% and that of hand-collected to 47% after 24 hours. Both types of samples were reduced to 0 germination after 5 days. Tests conducted with samples collected in 1954 showed that pollen stored in desiccators or vacuum desiccators at 0° F. did not retain its viability as well as pollen held in open containers at 0° F. It appeared that this difference was caused by the warming and opening of desiccators for removal of samples at each test date. The samples in open containers were removed individually, without disturbing the rest of the sample vials. Therefore, pollen collected in 1955 was either placed in vials with cotton plugs or in vials with aluminum foil-wrapped corks, containing two lumps of anhydrous calcium sulfate in the bottom. The latter acted as individual desiccators for small amounts of pollen. In this way, either desiccated or normal samples could be removed later for germination tests without disturbing the remaining sample vials. Results of germination test are presented in table 2. Note that desiccated samples retained considerably greater viability than the non-desiccated with the improved storage technique used in 1955. Bullock (1950) obtained 72% and 1% viable (hand-collected) pollen after 10½ months' storage at minus 5° F. with 25% and 75% relative humidities, respectively. Moisture apparently is an important factor in the frozen storage of either bee-collected or hand-collected pollen.

Although the hand-collected pollen maintained greater viability than the bee-collected pollen there were no detectable differences in pollen tube lengths or appearance in these tests. Germination tests were also conducted with pollen materials formulated with lycopodium spores at 30

Table 1.—Honey bee pollen-collecting activity observed in Washington apple orchards, 1954 and 1955.

	A.M.		P.M.		
	8–10	10–12	12–2	2–4	4–6
Percentage of pollen collectors[a]	39	33	24	18	9
Average number of pollen pellets trapped per hive	247	407	182	77	15

[a] As compared with total number of foraging bees on apple blossoms.

Table 2.—Viability of bee-collected and hand-collected pollen, Pullman, Washington, 1954–1956.

Sample Collected by	Type of Vials Used	Percentage Germination (No. Months Storage at 0° F.)					
		Initial	1	3	5	7	9
Bees, 1954	cotton-plugged	78	68	62	58	48	46
Bees, 1954	cotton-plugged in desiccator	78	72	64	58	50	32
Bees, 1955	cotton-plugged	81	70	63	54	50	45
Bees, 1955	corked, containing desiccant[a]	81	75	69	68	65	64
Hand, 1954	cotton-plugged	89	92	87	79	75	71
Hand, 1954	cotton-plugged in desiccator	89	88	86	77	76	69

[a] Individual desiccators.

Table 3.—Viability of bee-collected pollen dusts formulated with lycopodium, Pullman, Wash., 1955.

Treatment (Stored 11 Months)	Initial Per Cent Germination	Storage Temperature of Dust	Per Cent Germination (Storage Period) 5 Days	14 Days	14 Days +4 Hrs. at 75° F.
At 0° F.	45	0° F.	47	26	9
		35° F.	26	20	1
At 0° F. in dessicator	43	0° F.	44	25	31
		35° F.	29	15	1
At 0° F. (*not* formulated)	45	35° F.	16	16	5

pellets per gram of spores. Results of these tests are shown in table 3. The formulated dusts were difficult to wet with the usual 15% sugar solution. Therefore, a small amount was brushed onto each petri plate with a camel's-hair brush and then moistened.

RESULTS OF POLLINATION TESTS.—In 1954, eight limbs on Stayman Winesap trees were enclosed in screen sleeve-cages. Twenty-five blooms in each of four cages were hand-pollinated with bee-collected pollen. Twenty-five in each of the other four were not pollinated. The dust was formulated with powdered skim milk. About a month later, the fruit set was checked and the following results obtained: hand-pollinated, 5%; not pollinated, 0%; and normal (not in cages), 40%.

The pollen materials used in 1955 were formulated with lycopodium spores. This was easy to do with small amounts by "rolling" the substances together with a glass rod. A Stayman Winesap tree had been previously covered with a mosquito net cage for this work (fig. 1). All of the blooms on four limbs were hand-pollinated with one type of pollen dust. Three types of dusts and one untreated check were compared, using a total of 16 limbs. An added check was obtained from four limbs on an adjacent Stayman Winesap tree outside of the cage. All open blooms were pollinated on three different days, 2 to 3 days apart, in an effort to obtain maximum results. The data obtained in this test are presented in table 4.

Cool, windy weather limited honey bee activity in the experimental orchard during the blooming period. Very few bees were observed foraging the non-caged apple blossoms at that time and the nearest hives were about ½ mile from the orchard. However, the insect-pollinated check tree set far more fruits than the caged tree. The hand-collected pollen produced about 3½ times greater proportion of fruits per total blossoms than the bee-collected pollen. Bee-collected pollen stored for 1 year at 0° F. caused only about 2% less fruit set than the 2-weeks-old material. Poor fruit sets by means of bee-collected pollen were not due to incomplete mixing of the dust formulations, since microscopic examination showed that the pollen grains and lycopodium spores were well mixed.

CONCLUSIONS.—Use of bee-collected pollen for artificial pollination of apples is limited by the conditions necessary for collection. Large plantings of a suitable variety without competing pollen plants are not only desirable but also vital. Hand-collected pollen retained 71% viability, as shown by germination tests, after 9 months' storage at 0° F. in cotton-plugged vials. Bee-collected pollen maintained 46% viability under the same conditions. Bee-collected pollen samples stored in individual desiccators retained 64% viability after 9 months at 0° F. Data from the desiccator vs. non-desiccator tests, and data from Bullock's (unpublished) tests using different relative humidities, indicated that the viability of bee-collected and hand-collected pollens has an inverse relationship to the atmospheric moisture content of frozen storage containers. Normal insect pollination caused about 50% greater fruit set of Stayman Winesap than hand pollination with hand-collected pollen. Hand pollination with hand-collected pollen caused about 250% greater fruit set than hand pollination with bee-collected pollen. Germination tests indicated that the viability of the bee-collected pollen was more than adequate for pollination; however, there were very few fruits set with this type of material. Although many of the pollen grains were able to produce pollen tubes on an agar medium, they had become weakened in some way during storage. Apparently they were unable to penetrate through the style to successfully fertilize the ovules.

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Table 4.—Fruit set by means of hand pollination with bee-collected and hand-collected pollen, Pullman, Washington, 1955.

Source of Pollen	Fruit Set per Total Number Blooms per Limb: 1 Set	1 Total	2 Set	2 Total	3 Set	3 Total	4 Set	4 Total	Percentage of Total Blooms Set
Bee-collected, 1-yr.-old	4	91	5	78	4	82	1	76	4.3
Bee-collected, 2-wk.-old	11	130	3	166	0	17	13	101	6.5
Hand-collected, 2-wk.-old	20	66	25	119	4	37	8	30	22.6
Untreated check	1	91	0	144	0	96	0	65	0.3
Insect-pollinated check[a]	17	67	37	152	91	200	23	68	34.5

[a] Data from four limbs on a Stayman Winesap tree adjacent to the one in the test cage.

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Control of Green June Beetle Adults with Insecticides[1]

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The green June beetle, *Cotinis nitida* (L.), is found abundantly in the Eastern United States south of a line stretching from New Jersey to southern Illinois. The larvae often injure seedling tobacco and seedling legumes by uprooting these plants through their burrowing activities. The adults, like the adult Japanese beetle, have a preference for ripening fruits such as grapes and peaches. Both the larval and adult green June beetles have long been recognized as destructive pests in Kentucky. Garman (1904) states that: "The common green June bug, well known to every Kentucky school boy, becomes very troublesome locally and occasionally by cutting the skins of grapes and utterly destroying the fruit of whole bunches and even whole vines. On the Experiment Station Farm at Lexington this pest would, if allowed to work unhindered, destroy the whole crop of the early varieties in the experimental vineyard."

Although the control of the larvae has been studied extensively and many of the newer synthetic organic insecticides such as parathion, aldrin, lindane, heptachlor, and dieldrin have been shown to be very effective larvicides (Howe & Campbell 1953), there has been little recent research on the control of the adults with these insecticides. Most recommendations for their control suggest trapping the adults, shaking or jarring the beetles off the vines or branches into pans of kerosene, and bagging the fruit. The use of insecticides has usually not been recommended, probably because the time interval between application and harvest was thought to be too short to dissipate harmful residues.

During the summer of 1955, adult green June beetles were unusually abundant in Kentucky. Some varieties of grapes grown on the Experiment Station farm at Lexington were stripped of fruit and others so badly attacked that yields were substantially reduced. This infestation occurred long enough before the first harvest of grapes so it seemed probable that the application of some insecticidal sprays might not present a residue hazard. In order to determine which insecticides should be used in field experiments, a series of laboratory tests were conducted with 18 of the newer ones.

METHODS.—*Laboratory tests.*—The adult beetles were collected from grapes on the Experiment Station farm and kept in 2×2×2 foot screen cages in the laboratory until testing time. They readily fed on ripe grapes which were introduced as needed, and there was practically no mortality of the beetles while in these cages. The beetles were not separated as to sex in any of the tests.

The insecticides used were formulated from technical material with acetone as the solvent. One-half pint cardboard ice cream containers with the top replaced by organdy screen were used as test cages. Five milliliters of the formulated insecticide were spread uniformly over all inside surfaces of the container except the top. After the insecticide solution had dried, five, eight, or ten beetles, depending on the test, were placed in each treated cage. One or two untreated crushed grapes were placed in each cage at the start of the experiment and subsequently as needed. Because green June beetles often feign death, they were not counted as dead unless they showed no movement of any appendages when they were physically stimulated by blowing upon them.

Field experiment.—A heavily infested vineyard, consisting of mixed varieties of grapes, on the Experiment Station farm at Lexington, was used for a field test on the control of green June beetle adults. A randomized complete block design with seven replicates per treatment was used. Each plot consisted of 30 feet of a single row of grape arbor with 8 feet between rows. Some varieties of grapes, especially the early ripening French hybrids, were more severely attacked than others. The Concords were nearly completely free from infestations even when ripe, and the beetles were found feeding on some varieties which were still green in preference to the Concords.

The plots were sprayed on August 11 from 3:00 to 5:30 P.M. The temperature at the time of spraying was 80° F. and the wind velocity from 3 to 5 miles per hour. All the insecticides used were commercially prepared wettable powder formulations. The grape foliage and fruit were sprayed from both sides of the vines with a 2-gallon compressed air sprayer with a single Teejet No. 6504 nozzle until run-off occurred.

Counts of dead and live beetles were made 12 hours, 36 hours, and 7 days after treatment. Dead beetles were collected and counted along the 30 feet of row of each plot

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Table 1.—Laboratory test for the control of green June beetle adults.

Insecticides[a]	Per Cent Mortality at Specified Hours After Treatment[b]				
	6	18	24	32	48
Lindane	75	75	100	100	100
Heptachlor	10	85	85	100	100
Aldrin	0	50	85	90	95
Endrin	0	25	85	90	100
Isodrin	5	10	55	100	100
Dieldrin	0	0	30	65	100
Malathion	15	30	30	30	45
Toxaphene	0	5	5	10	75
DDT	0	5	10	15	15
Acetone check	5	5	5	15	20

[a] All insecticides at a concentration of one part per 10,000 parts of acetone.
[b] Four replicates for each treatment with five beetles per replicate.

to a width of 2 feet on either side of the grape arbor. All dead beetles were removed after each count. The live beetles were counted on the foliage and fruit along the entire length of each plot.

Two-pound samples of Concord grapes were collected for residue analyses from all treatments 1, 4, and 7 days after treatment. The grapes were placed in pliofilm bags, quick-frozen, and kept at −20 F. until all the samples had been taken. Samples of treated and of untreated grapes were then packed in dry ice and shipped by air express to the appropriate insecticide manufacturers for analyses.

RESULTS.—*Laboratory tests.*—In the first two laboratory tests on green June beetle adults 18 insecticides were tested at a concentration of one part of the insecticide to 10,000 parts of acetone. There were four replicates of each treatment with five beetles per replicate. Lindane, heptachlor, aldrin, and endrin gave 85% control or better 24 hours after treatment (table 1). At the same time interval, isodrin and parathion gave 55 and 50% control, respectively (table 2). All the phosphate insecticides, except parathion, gave unexpectedly poor control. None of the other materials gave better than 30% control 24 hours after treatment. Lindane, in these and subsequent tests, gave faster knock-down than any of the other materials tested. Six hours after treatment all the beetles in the lindane-treated cages were dead or visibly affected.

Table 2.—Laboratory test for the control of green June beetle adults.

Insecticides[a]	Per Cent Mortality at Specified Hours After Treatment[b]		
	6	16	24
Lindane	95	95	100
Parathion	0	20	50
Diazinon	0	0	10
Chlordane	0	0	5
Chlorthion	0	0	5
Bayer L13/59	0	0	0
Methoxychlor	0	0	0
TEPP	0	0	0
AC 528[c]	0	0	0
Acetone check	0	0	0

[a] All insecticides at a concentration of one part per 10,000 parts of acetone.
[b] Four replicates for each treatment with 5 beetles per replicate.
[c] 2,3-*p*-Dioxane dithiol S,S-bis(*O,O*-diethyl phosphorodithioate). Manufactured by Hercules Powder Co.

Table 3.—Comparison of lindane, heptachlor, and aldrin for the control of green June beetle adults in a laboratory test.

Insecticide	Concentration[a]	Per Cent Mortality at Specified Hours After Treatment[b]		
		16	24	48
Lindane	1:10,000	90	100	100
	1:20,000	80	100	100
	1:40,000	77.5	95	100
Heptachlor	1:10,000	62.5	90	97.5
	1:20,000	25	72.5	97.5
	1:40,000	10	72.5	95.0
Aldrin	1:10,000	40	85	97.5
	1:20,000	17.5	75	87.5
	1:40,000	2.5	32.5	67.5
Acetone check		0	2.5	12.5

[a] Insecticides diluted in acetone.
[b] Four replicates of each treatment with 10 beetles per replicate.

As lindane, heptachlor, and aldrin gave the fastest and highest mortalities, they were used in subsequent tests to determine more accurately their relative toxicities. Some of the other materials which appeared promising such as endrin, isodrin, and dieldrin, were not tested further because of their long residual property which it was thought would not fit into a spray program for green June beetle control on ripening fruit.

Lindane gave 100% mortality 48 hours after treatment at concentrations of 1:10,000, 1:20,000, and 1:40,000; heptachlor at the same levels gave nearly complete kill; but aldrin at 1:40,000 gave only 67.5% mortality (table 3). At all three time intervals at which counts were made lindane at 1:40,000 gave higher kills than heptachlor or aldrin at 1:10,000. In the next test lindane at 1:50,000, 1:75,000, and 1:100,000 gave excellent kills 24 hours after treatment but heptachlor and aldrin at 1:20,000 did not give as high mortalities as they did in the preceding test (table 4). These latter two insecticides did not act as fast as in the other tests and many of the beetles were knocked

Table 4.—Comparison of lindane, heptachlor, and aldrin for the control of green June beetle adults in a laboratory test.

Insecticide	Concentration[a]	Per Cent Mortality at Specified Hours After Treatment[b]			
		6	18[c]	24[c]	66[c]
Lindane	1: 50,000	32.5	100	100	100
	1: 75,000	22.5	100	100	100
	1:100,000	12.5	100	100	100
Heptachlor	1: 20,000	0	7.5	65	100
	1: 40,000	0	7.5	37.5	95
	1: 60,000	0	0	27.5	72.5
Aldrin	1: 20,000	0	0	45	92.5
	1: 30,000	0	5	37.5	92.5
	1: 40,000	0	0	40	85
Acetone check		0	0	0	5

[a] Insecticides diluted in acetone.
[b] Five replicates for each treatment with eight beetles per replicate.
[c] Dead and moribund beetles counted as dead.

Table 5.—Field test for the control of green June beetle adults on grapes at Lexington, Kentucky. 1955.

Insecticide (Pounds per 100 Gallons of Water[a])	12 Hours After Treatment[b]		36 Hours After Treatment[b]	
	Live	Dead	Live	Dead
Lindane, 0.25	0	146	3	61
0.125	0	166	26	53
Heptachlor, 0.5	0	67	47	31
0.25	20	16	50	15
Aldrin, 0.5	11	15	14	5
0.25	32	13	89	10
Untreated check	16	5	34	2

[a] Applied August 11.
[b] Totals from seven replicates of each treatment.

Table 6.—Analyses of grapes for residues of aldrin and heptachlor.

Insecticide	Pounds of Toxicant per 100 Gallons of Water	Residues on Specified Days After Application (P.P.M.)		
		1	4	7
Heptachlor	0.25	0.000	0.003	0.006
	.50	0.003	0.004	0.004
Check (heptachlor)[a]		0.003	0.001	0.002
Aldrin	.25	<0.1	<0.1	<0.1
	.50	<0.1	<0.1	<0.1
Check (aldrin)[b]		<0.03		

[a] Apparent heptachlor in check samples.
[b] Apparent aldrin in check samples.

down but not killed at both the 24- and 66-hour counts. When the dead and moribund beetles were counted together the results were very close to those obtained from the preceding tests.

It is evident from these laboratory tests that lindane affected the green June beetle adults more rapidly than the other insecticides tested, and was several times more toxic than heptachlor or aldrin. Although several chlorinated hydrocarbons were effective, none of the phosphorus insecticides, except possibly parathion, was promising for the control of this insect.

Field test.—For the same reasons that lindane, heptachlor, and aldrin were tested further in the laboratory, these three insecticides were used in a field experiment. Heptachlor and aldrin were applied at a concentration of 0.25 and 0.50 pound of actual toxicant in 100 gallons of water. Lindane was used at concentrations of 0.125 and 0.25 pound in 100 gallons of water. Counts of living and dead beetles made 12 hours after treatment indicated that lindane at both rates and heptachlor at the higher rate gave excellent control of the green June beetle adults as no live beetles were found in any of the plots (table 5). Heptachlor at the rate of 0.25 pound and aldrin at the rate of 0.50 pound gave fair control, but 0.25 pound of aldrin was not effective. Thirty-six hours after treatment both concentrations of lindane still gave good control; heptachlor at 0.5 pound gave fair to poor control; the other insecticidal sprays were ineffective. Seven days after application none of the treatments was effective and numerous live beetles were found in all of the treated plots. The field results were very similar to those obtained in the laboratory. Lindane was much superior to heptachlor and aldrin, and heptachlor was more effective than aldrin.

Residue Analyses.—The infestation of green June beetles on grapes in Lexington reached its peak at least 3 weeks before harvest. Results from residue analyses indicate that the time interval between insecticidal treatment and harvest may be long enough for some insecticides, which will effectively control the beetles, to be used without leaving harmful residues (table 6). Even 1 day after treatment the residues from either heptachlor or aldrin were less than one-tenth part per million.

Unfortunately, during the analysis for lindane, the lindane residues were lost and no insecticide was recovered. However, from a comparison of the residual action of lindane and that of either heptachlor or aldrin when applied to other fruits or vegetables, it would seem that the lindane residues would also be very low and well below the tolerance limits.

Summary.—Eighteen insecticides at a concentration of one part in 10,000 parts of acetone were tested in the laboratory to determine their toxicity to adults of the green June beetle. Lindane, heptachlor, aldrin, and endrin were found to be the most toxic of these materials, giving 85% or better kill 24 hours after treatment. None of the phosphorus insecticides was promising; parathion, which was the most toxic, gave only 50% mortality 24 hours after treatment. In further tests at lower concentrations, lindane proved to be much more toxic than either heptachlor or aldrin, and heptachlor was somewhat more toxic than aldrin. In a field test on a vineyard heavily infested with beetles, lindane at 0.125 and 0.25 pound per 100 gallons of water gave excellent control and heptachlor at 0.5 pound per 100 gallons gave good control. Heptachlor at 0.25 pound and aldrin at both 0.25 and 0.5 pound per 100 gallons of water gave poor results.

Residue analyses of the sprayed grapes indicated that heptachlor and aldrin would not leave harmful deposits from a spray program to control the green June beetle as less than 1 part per million of either insecticide was found 1 day after treatment. The lindane-treated grapes were sampled for residue tests but the lindane was lost during the process of analysis.

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The Influence of Colony Size and of Nosema Disease on the Rate of Population Loss in Honey Bee Colonies in Winter[1]

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While it is true that beekeepers may keep bees for several different purposes (for honey production, for production of package bees, or of queens, for educational purposes, for pollination, or simply as a hobby), and practical requirements may correspondingly vary, one aspect of the problem of wintering honey bees may, in its simplest terms, be stated as that of bringing the colonies out of winter with the number of bees reduced as little as possible below the autumn level—while at the same time avoiding disease. For honey production, Langstroth (1890), Farrar (1949) and others have advocated wintering really large colonies, while the theoretical viewpoint has been reached that the winter cluster is a more or less spherical heat unit which relies for its insulation not on the walls of the hive but on the outer layers of bees themselves (Phillips & Demuth 1914). The conception of such a heat unit is naturally a fertile field for ingenuity in the application of heat production and transfer calculations, and such ideas have been ably expressed, for example by Wedmore (1947). Calculations of this sort quickly lead to the conclusion that heat is more easily maintained by a large cluster than by a very small one, for it is from the sub-spherical surface of the cluster that the heat is lost, and the surface area does not increase proportionately with the number of bees, but only as the 2/3 power of the number—8 times the number of bees giving only 4 times the heat-dissipating surface. In general terms it would therefore be supposed that in winter the bees of a large colony would need to produce less heat per bee to maintain the minimum temperature necessary for healthy survival, and it would presumably follow that the strain on the individual bee during winter would for that reason be less in the larger colonies than in the smaller.

Thus most authors give the impression that increased colony size is a decided advantage, and though Wedmore does clearly show that for a given safe surface temperature the internal temperature will normally increase as the number of bees is increased, he does not suggest that there could be colonies so large as to make this a disadvantage, or that it could ever cause a much increased percentage of winter loss of bees, or cause restlessness and less satisfactory wintering.

The present paper, however, shows that such extra loss is a fact, and indicates that the losses in colonies of supernormal size become just as severe as those in very small colonies. This also shows that, using the term in its mathematical sense, there is an "optimum" wintering size (that is, a size giving minimum percentage loss of bees during winter), and that this proves to be well within the range of ordinary apiary practice.

Methods.—Over several years the numbers of bees in 153 queen-right colonies were determined in late autumn in an apiary in which considerable recurrence of nosema disease in spring was anticipated. Samples of 20 to 30 bees taken from the combs of all the colonies were tested by microscopical examination at the time of the autumn estimation and were found free from *Nosema apis*. Recently published data (Morison *et al.* 1956) have shown, however, that even in heavily infected apiaries *Nosema* disease is by no means easily found in October and November, when it reaches very low levels, often to reappear in a concentration much more easily detectable between February and July. The colonies were wintered near Aberdeen without packing and for the most part in Modified National hives. In the spring the numbers of bees were estimated again, and further tests on bees taken in the same way showed that 63 colonies had remained healthy while 90 were affected by *Nosema* disease. That the two groups of colonies thus selected were effectively different in respect of this disease was further substantiated by the subsequent microscopical tests on bees from 43 of the healthy colonies, made 1 month or more after the spring estimation, when they were still found to be free from *Nosema*. In 21 instances a third test was made before the end of June, and again proved negative. The records cover the period autumn 1944 to spring 1954 in the case of the healthy colonies, and autumn 1947 to spring 1954 for those which developed *Nosema* disease. There was no acarine disease in these experimental colonies.

Two methods were used for estimating colony strengths: (1) A visual method of comparing the number of bees on each comb in turn with accurately calibrated photographs. For a colony of about 10,000 bees this method has been shown to give an estimate with a standard deviation in the region of 7.6% of the true value (Jeffree 1951); (2) by a weighing technique (Jeffree 1955), which would certainly be appreciably more accurate. Out of the total of 306 estimations, 61 were made by the visual method only, 228 by weighing only, and 17 by both methods.

The majority of estimations were carried out in November and late March, but owing to cold weather and other eventualities the number of days between the two estimations necessarily varied from year to year, and also between different colonies in the same year. In every case, therefore, the spring colony size has been corrected by a logarithmic method to a uniform period of 150 days, the mean time between the autumn and spring examinations. The estimate in logarithmic form of the ratio of autumn strength to spring strength adjusted to 150 days is given by

$$\frac{\text{log. autumn size} - \text{log spring size}}{\text{actual no. of days between estimations}} \times 150$$

This figure subtracted from log. autumn size gave the corrected log. spring size. The antilogarithm of the corrected value was used in all the calculations, and is referred to hereafter as the spring size.

Results.—Corresponding autumn and spring sizes of

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healthy colonies and of those with *Nosema* disease are shown in figures 1 and 2, respectively. It can be seen that in each group the points lie about a curve. However, if spring size is plotted against $\log_{10}$ autumn size the relationship is very nearly linear in both cases (fig. 3). The slopes of the regression lines for the two groups of colonies were not significantly different and since a combined regression line was found to provide a more accurate estimate of the slope of each line individually it has consequently been given in figure 3. The scatter of points about the line did not differ significantly for the two groups.

Writing y for spring size and x for $\log_{10}$ autumn size, the regression equations are:

$$\text{Healthy: } y=(21{,}040\pm1{,}070)x-76{,}130$$
$$\text{Nosema: } y=(21{,}040\pm1{,}070)x-77{,}880$$

The regression lines in figure 3 have been transformed and plotted in figures 1 and 2 also.

Although the regression coefficients for the two groups of colonies were effectively the same, the difference between the absolute terms in the above equations (1,750 $\pm$500) was highly significant ($p<.001$). For corresponding autumn sizes, therefore, the healthy colonies were stronger by approximately 1,750 bees in the spring than those with *Nosema* disease. Moreover, the estimate of this figure for the extra loss occasioned by the disease is the same for all values of autumn size. The possibility of further loss in the *Nosema* colonies after the date of spring estimation has not here been investigated.

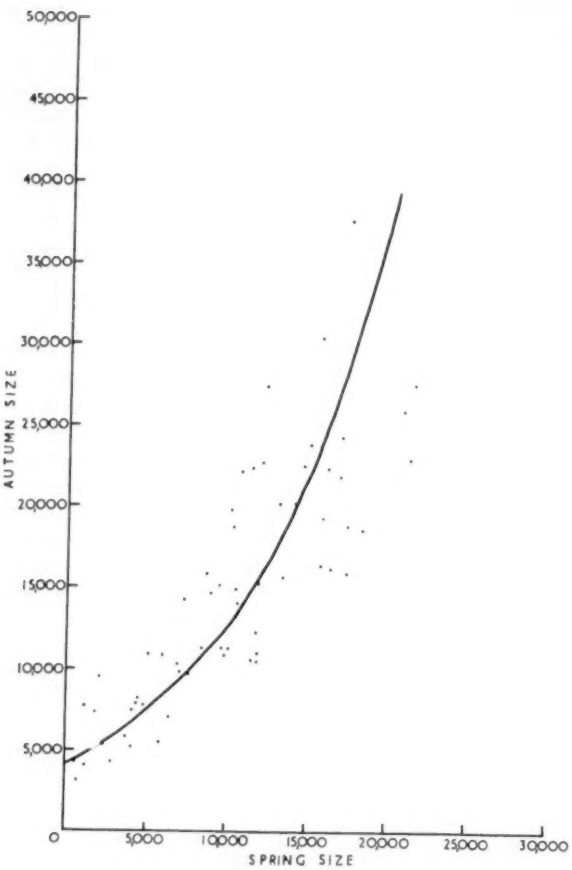

FIG. 1.—Winter losses in healthy colonies.

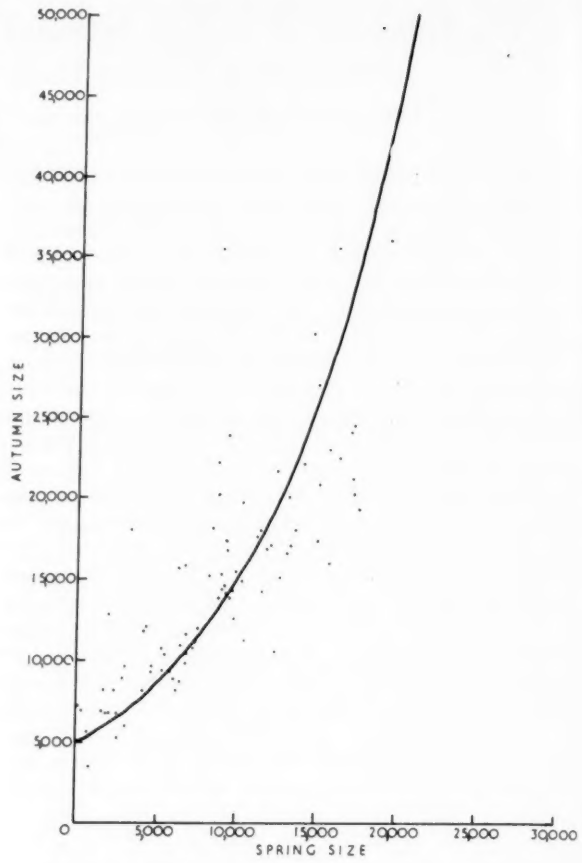

FIG. 2.—Winter losses in colonies with *Nosema* disease.

It appears that both in healthy colonies and in those with *Nosema* disease the proportional loss is greater for small and for very large colonies than for colonies of medium size. The "optimum" late autumn sizes (i.e. those giving the smallest relative winter losses of bees) correspond with the points at which tangents through the origins of figures 1 and 2 touch each curve. This "optimum" size is about 11,000 bees for healthy colonies, and about 14,000 bees for those affected by *Nosema*. For example, it can be seen that a healthy colony with an autumn size of 11,000 bees would be expected to lose 18% of its bees by the spring, while colonies with autumn strengths of 7,000 and 35,000 bees would lose 36% and 44%, respectively. Similarly, colonies with *Nosema* disease having autumn strengths of 7,000, 14,000 and 35,000 bees would suffer winter losses of 57%, 33% and 51%, respectively. These values for the "optimum" wintering sizes are not to be regarded as determined with any high accuracy from the data since they would be seriously affected by small changes in the coefficients, but there is no doubt that there really is an "optimum" size. Indeed a calculation taking into consideration the variability of the data shows that for the healthy colonies the "optimum" November size lies between 8,120 and 15,120 bees, and for the *Nosema* colonies between 10,700 and 19,050.

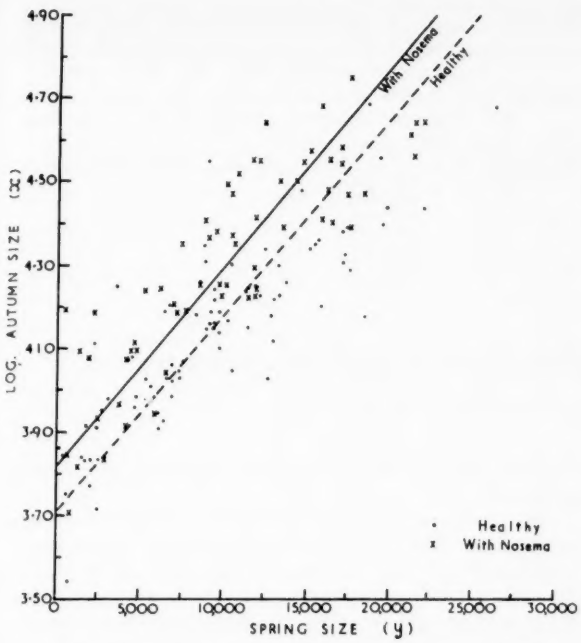

FIG. 3.—Regressions of spring size on $\log_{10}$ autumn size for healthy colonies and for colonies with *Nosema* disease.

The above limits could conveniently be regarded as upper and lower limits for wintering bees with minimum winter loss; but in this connection it must also be remembered that the corresponding sizes would be considerably larger if measured in early autumn instead of in November, as there is appreciable reduction in colony strengths in the autumn months. Unpublished data in this Department indicate that the approximate corresponding "optimum" sizes in August, September and October for healthy colonies would be 18,000, 15,000 and 13,000 bees, respectively, while for those expected to develop *Nosema* disease the "optimum" sizes in these three months would be respectively 22,000, 19,000 and 16,000 bees.

DISCUSSION.—The general finding that very large colonies suffer heavy loss equally with very small colonies is surprising, and no data are here brought forward to indicate the cause of this clear effect. It is, however, evident from the mathematics of heat distribution and transference within the cluster that as the cluster size increases the central temperature necessarily becomes higher for any given value of the surface temperature. It is therefore, suggested as a probable explanation that in a very large cluster the bees have some difficulty in keeping the center of the cluster cool enough while at the same time retaining a sufficiently high surface temperature. Two ways would seem to be open to the bees in an attempt to do this: (1) to increase the heat conductivity of the bees in the cluster—this might be achieved by the hot bees at the center moving continually to the outside, which would seem to correspond with disturbed wintering; (2) to generate their heat all towards the edge of the cluster and none of it at the center. This would apparently correspond with the theoretically hollow shells of bees discussed by Wedmore (1947), and it is commonly accepted that the center of the cluster is less dense than the outer shell; but it is unlikely that there would ever be any large central part entirely without bees. Research, however, is clearly necessary to establish the actual factors involved in this increased loss of bees in very large clusters.

The results are for colonies which were in receipt of no special treatments. The quantities of pollen present were, for example, the small amounts normally found in queen-right colonies wintering at Aberdeen (for a discussion of these levels see Jeffree 1956), and no attempt was made to assess the extra effects of added pollen.

For some purposes beekeepers might require colonies either larger or smaller in spring than the sizes resulting from the above "optimal" wintering. Such colonies could be obtained in two distinct ways. Either (1) by wintering the colonies respectively as larger or smaller units, or (2) by wintering as "optimally" sized units and in spring uniting or, respectively, dividing. The present work suggests that of these two ways, method (2) would in general be more economical in bees than method (1). The present paper, however, is intended as a contribution to the theory of the loss of bees from over-wintered colonies, and does not presume to give advice on beekeeping methods.

Hitherto, detailed assessment of the damage caused by *Nosema* disease has not been available as a general statement for colonies of various sizes. It has been apparent in our work that although the disease causes considerable reduction in strength, it results in the death of relatively few colonies, the majority of those suffering this fate having been already small in the autumn. It is obviously important, therefore, in apiaries where the disease is expected that colonies should be of an adequate strength to withstand the additional losses likely to result.

ACKNOWLEDGMENT.—The authors wish to thank Dr. F. H. C. Marriott for statistical advice.

SUMMARY.—Records were made of the autumn and spring sizes of 63 healthy colonies between the autumn of 1944 and the spring of 1954. Similar estimations in the same apiary were also carried out between the autumn of 1947 and the spring of 1954 on 90 colonies with *Nosema* disease.

The curves relating autumn and spring colony sizes were of the same general shape in the healthy colonies and in those with *Nosema* disease. The diseased colonies, however, lost approximately 1,750 more bees than those which had remained healthy. This extra loss was significant, and was independent of the initial colony size.

The percentage loss of bees over winter was found to be significantly greater for very large and for very small colonies than for colonies of medium size. In this sense, the fact of a theoretically optimum size for wintering was thus established. This would be approximately 15,000 bees in September for healthy colonies, and 19,000 for colonies with *Nosema* disease. Corresponding sizes for other autumn months are indicated.

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Control of the European Corn Borer with Granulated Insecticides in 1955[1]

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In experiments conducted at Ankeny, Iowa, in 1953 and 1954 (Cox *et al.* 1956), granulated formulations of certain insecticides were found to be very effective for control of the European corn borer, *Pyrausta nubilalis* (Hbn.). This study was continued and expanded during 1955, to give particular emphasis to different insecticides, formulations, rates of application, and time of application, as well as machines used for the application of granules (Lovely *et al.* 1956) and residues on plants following treatment (Fahey *et al.* 1956).

General Procedure.—A reciprocating chain-type seeder was used to apply the granules in all experiments except where otherwise specified. A high-clearance sprayer, modified to treat four rows simultaneously, was used to apply the emulsions.

The plots were four rows wide by 140 feet long. Each treatment was replicated four times. Since the machines for applying granules were designed to treat only two rows, the two center rows from each four-row plot were used for observation.

The procedure for dissecting plants was the same as in the previous experiments. However, the larval instars were not recorded. Although the number of borer tunnels in each plant was recorded, these data are not included herein since statistical analyses showed a high correlation between borers and tunnels.

Yield estimates were obtained by a modification of the procedure reported by Cox & Lilly (1953). The sample area was the two center rows from the four-row plots, starting in the plots 10 feet from each end. The ear from every fifth plant that produced an ear was harvested. The ears from each plot were counted, weighed, and sampled for moisture determination. After correction of the field weights to 15.5% moisture, the yields were calculated.

Results.—The results of experiments on first-brood borer control only are reported. Several experiments on second-brood control were undertaken, but the data were of questionable value owing to a prolonged egg-deposition period and an insignificant borer population.

Insecticides.—Granulated formulations[5] of 14 insecticides were compared in a randomized complete block experiment. Whorl-stage corn was treated with the various materials on June 23. At that time all the plants showed leaf feeding by borers. The corn was Ia. 4298. A DDT emulsion spray was used as a standard of comparison.

The data obtained from this experiment are summarized in table 1. All the materials tested caused highly significant decreases in the borer population. The differences between the insecticides were also highly significant. Endrin, heptachlor, aldrin, and dieldrin were superior to Am. Cyanamid 3911 (Thimet), ryania, rotenone, and malathion. DDT granules were just as effective as the DDT spray and gave 87% control. Endrin, heptachlor, aldrin, dieldrin, isodrin, and parathion gave better control than the standard DDT treatment. Toxaphene and Strobane at 2 pounds per acre gave control almost as good as DDT at 1½ pounds.

Some of the treatments that gave the best control were sampled for yield. With the exception of the DDT-sprayed plots, the treated plots showed yield increases of 3 to 15 bushels per acre (table 1). Sampling error seemed to be the only explanation for the low yield in the DDT-sprayed plots. Good control of the borer caused an over-all yield increase of approximately 8 bushels per acre. However, at the 5% probability level, yields from the treated plots were no better than those from the untreated plots.

DDT and malathion emulsions and granules were compared in a split-split-plot experiment on sweet corn.[6] Ten per cent of malathion impregnated on 30/60-mesh Celite (diatomaceous silica) and 7.5% of DDT impregnated on 30/60-mesh RVM-AA attapulgite were each applied at the rate of 20 pounds per acre. The malathion emulsion was applied at the rate of 2 pounds and that of DDT at 1½ pounds of active ingredient in 10 gallons of water per acre. The first application was made on June

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[3] Agricultural Engineering Research Branch, Agr. Res. Serv., U.S.D.A.

[4] Iowa State College.

[5] The following companies supplied the formulations used in this study: American Cyanamid Co., B. F. Goodrich Chemical Co., Diamond Black Leaf Co., Hercules Powder Co., S. B. Penick and Co., Shell Chemical Corp., and Zonolite Co. Mackwin Co. prepared and furnished many of the special formulations.

[6] The authors appreciate the cooperation and interest shown by the Green Giant Canning Co. in furnishing the Green Giant Code 10 seed used to plant this field.

Table 1.—Effectiveness of various granulated insecticides against first-brood European corn borers, 1955.

Insecticide	Concentration (Per Cent)	Pounds of Active Ingredient per Acre	Carrier	Number of Larvae per 100 Plants	Yield (Bu./A.)
Endrin	2	0.4	Attapulgite	8.8	59.2
Heptachlor	7.5	1.5	Attapulgite	8.8	—
Aldrin	5	1	Attapulgite	15.0	54.7
Dieldrin	5	1	Attapulgite	20.0	57.2
Isodrin	5	0.4	Attapulgite	23.8	51.0
Dieldrin	2	0.4	Attapulgite	28.8	56.1
Endrin	2	0.4	Vermiculite	30.0	63.0
Parathion	5	0.5	Attapulgite	36.3	—
DDT	25	1.5	Emulsion spray	42.5	48.2
	7.5	1.5	Attapulgite	42.5	60.8
Toxaphene	10	2	Attapulgite	48.8	—
Strobane	25	2	Attapulgite	48.8	—
EPN	2.5	0.5	Attapulgite	52.5	—
Am. Cyanamid 3911	2	0.5	Celite	72.5	—
Malathion	10	2	Celite	72.5	—
Rotenone	5	1	Attapulgite	76.3	—
Ryania	40	8	Attapulgite	80.0	—
Am. Cyanamid 3911	20	0.5	Celite	103.8	—
Untreated	—	—	—	330.3	48.0

2, just after the beginning of egg laying but prior to hatching. Seven days elapsed between the first two applications, and 5 days between the others. Single, double, and triple applications were made. Table 2 is a summary of the results obtained. DDT was significantly better than malathion.

Formulations.—Analysis of the data in table 2 showed that the granulated formulation of either DDT or malathion gave borer control equally as good as the emulsion formulation of the same material.

Granules and emulsions of DDT were applied on different dates in a randomized complete block design experiment. The borer-susceptible double-cross hybrid Ia. 4297 was in the whorl stage at the time of the first treatment. The formulations and rates of application were the same as in the previous experiment. Both single and double applications were made. The data from this experiment, summarized in table 3, showed significantly better control with the granules than with the emulsion.

Carriers.—A hypothesis existed that hard particles would not adhere to the leaves in the whorls and hence would stay in the whorls while the leaves were growing out. It was also thought that a hard particle would release the toxicant more slowly; consequently, it seemed

Table 2.—Average numbers of first-brood European corn borer larvae per 100 sweet corn plants after treatment on different dates with DDT and malathion, 1955.[1]

Application Dates (June)	DDT		Malathion	
	Granules	Emulsion	Granules	Emulsion
2	115.0	137.5	171.3	148.8
9	92.5	95.0	146.3	178.8
14	51.3	62.5	65.0	167.5
2 and 9	65.0	76.3	145.0	201.3
9 and 14	73.8	51.3	92.5	117.5
14 and 19	27.5	25.0	78.8	65.0
2, 9, and 14	62.5	40.0	53.8	113.8
9, 14 and 19	50.0	27.5	42.5	71.3
14, 19, and 24	28.8	16.3	42.5	33.8

[1] Untreated plots had 161.3 larvae per 100 plants.

Table 3.—Average numbers of first-brood European corn borer larvae per 100 field corn plants and yield after treatment with DDT on different dates, 1955.[1]

Application Date (June)	Granules		Emulsion	
	Larvae	Yield (Bu./A.)	Larvae	Yield (Bu./A.)
2	238.8	61.2	333.8	56.2
9	165.0	60.4	163.8	62.5
14	141.3	66.5	141.3	59.3
19	85.0	60.0	71.3	56.2
24	16.3	68.1	55.0	63.3
2 and 9	130.0	66.3	192.5	65.0
9 and 14	112.5	64.2	138.8	62.3
14 and 19	57.5	65.3	27.5	66.8
19 and 24	12.5	62.7	40.0	61.4
24 and July 1	13.8	68.5	21.3	65.0

[1] Untreated plots had 413.8 larvae per 100 plants and a yield of 49.9 bushels per acre.

possible that hard granules might give an increase in borer control.

Four particle sizes of both RVM-AA and LVM-A attapulgite—15/30, 20/30, 30/40, and 30/60 mesh—were compared in a split-plot design experiment. The LVM-A material is approximately twice as hard as the RVM-AA. All particle sizes of both types of carriers were used to prepare granules containing 7.5% of DDT. The rate of application was 20 pounds of granules per acre. Approximately 90% of the plants were showing leaf damage when treatments were made on June 20. The corn in this and the following experiment was Ia. 4297.

The data, summarized in table 4, show that significantly fewer borers were found in the plots treated with the RVM-AA carrier than in plots treated with the LVM-A carrier.

A split-split-plot design experiment was used to compare application equipment, carriers, and particle size. The carriers were tobacco base, and RVM and LVM attapulgite. Particle sizes were 15/30, 30/40, and 30/60 mesh for all carriers but one. A 30/40-mesh tobacco formulation was not available and a 20/40-mesh formulation was substituted. The attapulgite carriers were available as 7.5% DDT formulations while the tobacco was a 10% formulation. Rates of application were 10 pounds of attapulgite and 7½ pounds of tobacco-base granules per acre. Treatments were made on June 20.

The data from this experiment, summarized in table 5, showed highly significant differences among carriers. RVM-AA attapulgite was superior to tobacco base, which in turn was superior to LVM-A attapulgite. How-

Table 4.—Effect of particle size and type of attapulgite granules on control of first-brood European corn borer larvae with 20 pounds of 7.5% DDT, 1955.

Particle Size (Mesh)	Larvae per 100 Plants	
	RVM-AA	LVM-A
15/30	80.0	116.3
20/30	58.8	151.3
30/40	105.0	118.8
30/60	102.5	97.5
Untreated	513.8	

Table 5.—Effect of equipment, carrier, and particle size on the control of first-brood European corn borer larvae with DDT granules, 1955.[1]

Carrier and Particle size (Mesh)	Chain-Type Seeder		Fluted-Shaft Seeder		Fluted-Feed Seeder	
	Larvae per 100 Plants	Yield (Bu./A.)	Larvae per 100 Plants	Yield (Bul/A.)	Larvae per 100 Plants	Yield (Bu./A.)
Attapulgite, RVM-AA						
15/30	106.3	40.2	78.8	54.1	57.5	53.7
30/40	170.0	47.0	46.3	51.9	43.8	65.0
30/60	61.3	44.1	76.3	63.0	42.5	56.8
Attapulgite, LVM-A						
15/30	112.5	—	170.0	—	122.5	—
30/40	91.3	—	182.5	—	86.3	—
30/60	103.8	—	175.0	—	82.5	—
Tobacco base						
15/30	108.8	—	162.5	—	113.8	—
20/40	108.8	—	105.0	—	98.8	—
30/60	92.5	—	117.5	—	98.8	—

[1] Untreated control plots had 292.5 borers per 100 plants and a yield of 43.7 bushels per acre.

ever, there was a significant insecticide-carrier by application-equipment interaction. Poorer control was obtained with RVM-AA attapulgite when applied with the chain-type seeder than when applied with the fluted-shaft seeder or the fluted-feed machine. On the other hand, poorer control was obtained with LVM-A attapulgite when applied with the fluted-shaft seeder than when applied with the chain-type seeder or the fluted-feed machine.

Different types of carriers and types of machines were compared in a split-plot design experiment. The carriers tested were as follows: RVM-AA attapulgite, 30/40 and 30/60 mesh; RVM-AA attapulgite, 30/40 mesh with adjunct added to impregnating solvent; two types of tobacco base, sterilized and specially extracted; sodium bentonite, hygroscopic and easily dispersible in water; perlite, a heat-expanded volcanic material; Celite, calcined diatomaceous earth; and vermiculite, heat-expanded mica. All the materials were prepared as 4% DDT formulations and applied at the rate of 20 pounds of granules per acre. Approximately 95% of the plants were showing leaf damage when the treatments were applied on June 21. The corn was Ia. 4298.

Results of this experiment, summarized in table 6, showed no differences in borer control due to different machines. Lovely *et al.* (1956) discussed the mechanical behavior of carriers in relation to application equipment. All the treatments caused a highly significant reduction in the borer population below the untreated control. When the plots treated with attapulgite, tobacco-base, and bentonite carriers were compared with those treated with perlite, Celite, and vermiculite carriers, the difference was significant at the 1% level. Borer control in plots treated with the tobacco-base and attapulgite granules averaged 75 and 81%, bentonite and perlite 71 and 68% control, respectively, and Celite and vermiculite 61 and 53%.

Analysis of the yield data showed significant differences between the yields obtained with the different machines. Plots treated with the chain-type seeder had the highest total yield. Yields for the fluted-shaft seeder and the fluted-feed machine were about equal.

Yields from the treated plots were significantly higher than those from the untreated plots. There was no difference at the 5% level between plots treated with the different carriers.

Particle Size.—It seemed possible that granules slightly larger than 30/60 mesh would be less affected by air turbulence. An analysis of the data summarized in table 4 showed no significant difference in effectiveness due to particle size. The 15/30-mesh particles were just as effective as 30/60-mesh particles, although a unit weight of 30/60 particles contained approximately 10 times as many particles as an equal weight of 15/30 particles. However, in the experiment summarized in table 5 there was a significant difference between granules of different sizes. Fewer borers were found in plots treated with 30/60-mesh granules than in plots treated with the larger granules.

Only the plots treated with RVM-AA attapulgite were sampled for yield estimates. There was no relation be-

Table 6.—First-brood European corn borer control using three types of equipment to apply different types of DDT-impregnated carriers,[a] 1955.

Carrier	Particle Size	Chain-Type Seeder		Fluted-Shaft Seeder		Fluted-Feed Seeder	
		Larvae per 100 Plants	Yield (Bu./A.)	Larvae per 100 Plants	Yield (Bu./A.)	Larvae per 100 Plants	Yield (Bu./A.)
Attapulgite, RVM-AA with adjunct	30/40	37.5	59.8	27.5	57.0	35.0	62.4
Attapulgite, RVM-AA	30/40	30.0	61.5	30.0	53.1	57.5	53.0
	30/60	37.5	68.9	38.8	55.4	33.8	52.1
Tobacco base:							
Sterilized	30/60	36.3	59.1	50.0	61.3	41.3	50.0
Specially extracted	30/60	41.3	60.5	58.8	58.4	32.5	56.1
Bentonite	30/40	67.5	62.5	25.0	65.0	58.8	66.4
Perlite	20/40[b]	41.3	63.1	57.5	54.5	66.3	46.1
Celite	30/60	58.8	61.2	61.3	54.1	82.5	60.9
Vermiculite #4	20/40[b]	46.3	58.3	106.3	51.7	95.0	58.6
Untreated		153.8	54.0	170.0	48.0	197.5	52.7

[a] DDT applied at rate of ¼ pound per acre.
[b] Approximate.

tween yield and particle size except in the plots treated with the chain-type seeder. Comparison of the treated and untreated plots did not show yield increases that were significant at the 5% level.

Rate of Application.—When granules were found to be effective for borer control, it seemed desirable to determine the most practical rate of application. The standard recommendation for DDT in emulsions is 1½ pounds per acre (Entomology Research Branch 1955).

Four concentrations of DDT on RVM-AA attapulgite —4, 7.5, 15, and 30%—were used in a split-plot design experiment. The rates of application were intended to be 20, 10, and 5 pounds of granules per acre, but the speed of the duster was unavoidably altered and the rates actually obtained were 12.6, 6.3, and 3.2 pounds per acre. The materials were applied to Ia. 4297 in the whorl stage on June 20, when about 90% of the plants showed leaf damage.

Analysis of the data, summarized in table 7, showed significant differences in control between the rates of application as well as between concentrations. The 12.6-pound rate was superior to 6.3 pounds, which in turn was superior to 3.2 pounds. The 30 and 15% DDT formulations gave better control than those containing less DDT. However, these data combined with other experiments reported herein suggest that 15 to 20 pounds of a 4 to 7.5% DDT formulation would give the most practical and economical corn borer control. All the treated plots had corn yields greater than the untreated plots. The largest increase, 12 bushels per acre, occurred in the plots treated with 12.6 pounds of granules. The yield increases within a particular rate of application were not directly related to reduction in borer numbers. There were no significant differences in yields between the treated plots.

Time of Application.—It is known that control with emulsion sprays is dependent on their time of application. However, since granules tend to fall deeper into the whorl leaves than sprays, their time of application might not be as critical simply because the toxicant would remain nearer the primary point of feeding for a longer time. Figure 1 shows a granulated insecticide in the moist area of the whorl leaves where the larval population usually becomes established. On sweet corn there were highly significant differences in the control obtained on different dates of application (table 2). The best single application was made on June 14, the best double application on June 14 and 19, and the best triple application on the same dates and June 24. These data indicate that the best control was obtained from multiple applications when egg laying was practically completed and from 42 to 98% of the plants were showing leaf damage (fig. 2).

FIG. 1.—Whorl leaves of a corn plant with a granulated insecticide adhering to the area of moisture. The moist area is the primary point of feeding of newly hatched borer larvae.

Table 7.—Average numbers of first-brood European corn borer larvae per 100 plants and yield after treatment with granulated DDT at different rates and concentrations, 1955.

RATE OF APPLICATION (LB./A.)	PER CENT DDT	LARVAE	YIELD (BU./A.)
12.6	4	103.8	56.5
	7.5	96.3	63.0
	15	50.0	57.5
	30	48.8	59.5
6.3	4	156.3	55.8
	7.5	145.0	56.2
	15	117.5	54.2
	30	95.0	53.7
3.2	4	253.8	57.0
	7.5	200.0	54.0
	15	230.0	57.6
	30	146.3	61.9
Untreated	—	433.8	47.2

On field corn double applications of both emulsion and granules were significantly better than single applications, and both single and double treatments were significantly better when applied late (table 3). As shown in figure 2, 84% of the plants showed leaf damage on June 19 and 100% on June 24. It will be noted that leaf damage reached 100% 2 days before the best single-application control was obtained. Usually the best control with a single application of an insecticide is obtained when from 75 to 90% of the plants show leaf damage. However, in 1955 extremely cool weather from June 8 to 13 seriously depressed the borer population. Consequently, there was no means of showing whether leaf damage was due to borers feeding in the whorls or to borers later killed by cold weather. This situation naturally led to a higher percentage of the plants showing leaf damage than was actually caused by the subsequent borer population.

The average yield was slightly over 63 bushels of corn per acre in the treated plots and slightly less than 50 in the untreated plots. This difference was significant at the 1% level. The average yield in plots treated with

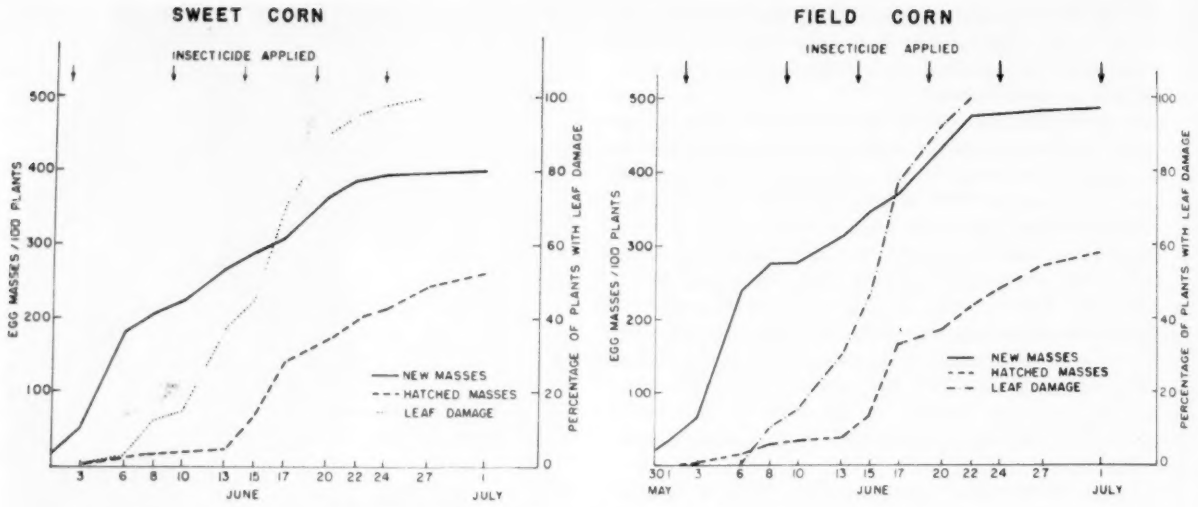

FIG. 2.—Time of insecticide applications in relation to corn borer egg laying, hatching, and leaf-feeding damage, Ankeny, Iowa, in 1955

granules was significantly higher than in the sprayed plots. The higher yield was especially noticeable in plots with a single application. The granule-treated plots had from 3 to 4 more bushels of corn per acre than the sprayed plots. No significant increase in yield was found in the plots which exhibited the best borer control (early applications) over plots showing poorer control (late applications).

SUMMARY AND CONCLUSIONS.—Granulated formulations of insecticides for control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) were studied at Ankeny, Iowa, for the third year in 1955. Experiments with the first-brood larvae are presented here.

Endrin, heptachlor, aldrin, dieldrin, isodrin, and parathion granules gave better borer control than either granulated or spray formulations of DDT. DDT at $1\frac{1}{2}$ pounds per acre was only slightly better than toxaphene and Strobane at 2 pounds. DDT was better than malathion.

Granulated formulations of DDT were just as effective as spray formulations.

As insecticide carriers in granulated formulations, RVM-AA attapulgite, tobacco-base, and bentonite were superior to perlite, Celite, vermiculite, and LVM-A type attapulgite.

One experiment showed no difference in borer control due to the size of the granules, but in another experiment significantly better control was obtained with 30/60-mesh than with larger particles.

High rates of application of granules of low DDT concentration gave better kills than low rates of application of granules of high DDT concentration. On a practical basis 15 to 20 pounds of a 4 to 7.5% DDT formulation will give the best borer control.

Double or triple applications of insecticides were more effective than single applications. The best time for a single treatment was when egg laying was practically completed and 75 to 90% of the plants showed leaf-feeding damage by borers.

Properly timed applications of insecticides in all experiments resulted in an average yield increase of almost 10 bushels of corn per acre.

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Application Equipment for Granulated Insecticides[1]

W. G. Lovely,[2] H. C. Cox,[3] and T. A. Brindley[3,4]

Granulated insecticides have been successfully used to control corn earworm and corn rootworm (Farrar 1953) and mosquitoes (Gooden 1953). Results obtained from research conducted in 1953 and 1954 at the European Corn Borer Research Laboratory in Ankeny, Iowa, showed promising results for the use of granulated insecticides to control the European corn borer reported by Cox *et al.* (1956). It was carried on cooperatively by the U. S. Department of Agriculture and the Iowa Agricultural Experiment Station. These promising results led to the expansion of the research program in 1955.

General Procedure.—Machinery investigations were divided into three parts. First, the preliminary laboratory studies were conducted to determine some of the physical properties of the granulated carriers and how these properties affected the functioning of different metering mechanisms. Secondly, field experiments were conducted using three different machines for first brood borer control. Thirdly, field experiments were conducted using five different machines for second brood borer control. For the laboratory studies five machines with different types of metering mechanisms and five different carriers of several different sizes were selected. Volume weights and angle of repose were obtained for the various materials used. The physical breakdown of the granulated carriers as they were metered through some of the machines was also determined.

Field plots for both first and second brood borer studies were four rows wide and 140 feet long. Randomized block and split-plot design experiments with untreated checks and four replications were used. The larvae counts and tunnel counts were obtained in the same manner as was reported by Cox *et al.* (1956). Yield data were obtained by sampling an area in the two center rows from each of the four row plots. The ear from every fifth plant that produced an ear was harvested and yields were converted to bushels per acre at 15.5% moisture. Residue samples were obtained for first brood studies by collecting 10 corn plant whorls from each plot. For second brood work the samples included stalks, leaves, and axils. Residue analyses were reported by Fahey *et al.* (1956).

Objectives.—The objectives of the engineering phases of this work were as follows: 1, to study the physical properties of the granulated carriers and the effect they had on machine functioning; 2, to test several types of machines in field experiments; 3, to study the various types of metering mechanisms that could effectively and efficiently apply granulated insecticides; and 4, to obtain engineering information that would aid in the development of commercial equipment for applying granules.

Results.—The results will be discussed in three parts: namely, preliminary laboratory tests, field studies of first brood borers, and field studies of second brood borers.

Preliminary Laboratory Tests.—A variable-speed drive stand was constructed to operate the various machines in the laboratory. An auger-type fertilizer distributor,[5] a fluted-feed grass seeder,[6] a reciprocating-chain type grass seeder,[7] a fluted-shaft granular applicator,[8] and a reciprocating-rope seeder[9] were tested using carriers without toxicants to determine the metering characteristics.

The attapulgite, tobacco, bentonite, and vermiculite[10] were selected as representative samples of the carriers that would be used in 1955.

Table 1 shows the breakdown of particle size that occurred when these carriers were run through the different machines. The per cent breakdown of any given material for the various machines was not serious.

Table 1.—Physical breakdown of granules in per cent of fines[a] after metering.

Carrier	Size	Per Cent Below No. 60 Sieve				
		Un-Metered[b]	Reciprocating Rope	Reciprocating Chain	Fluted Shaft	Fluted Feed
Attapulgite RVM	30/60	5.5	6.0	6.4	7.3	9.1
Attapulgite RVM	15/30	1.0	1.7	2.9		
Attapulgite RVM	8/20	1.0	4.0	1.7		
Attapulgite LVM	15/30	1.0	2.0	1.2		
Bentonite	30/40	3.4	3.7	5.7		
Tobacco	30/60	2.0	2.5			
Vermiculite	30/60[c]	13.4		14.5		

[a] Material that will pass through a No. 60 sieve.
[b] A representative sample was run through a nest of sieves with a roto-tap shaker for 5 minutes.
[c] Approximate.

As shown in table 2 the volume weights of the carriers plus the insecticides as used in field tests vary from 14.4 to 73.0 lbs. per cu. ft. This indicates that the metering mechanism of any given machine needs a wide range of discharge capacity. For a given number of pounds per acre it would take nearly seven times the volume for the lighter material as it would the heavy material. These data also give indications to the size and strength of hoppers that could be used for these various materials.

The angle of repose was determined to provide an index to the flowability of the various carriers. These data indicate that it is necessary for gravity feed hoppers

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[2] Agricultural Engineering Research Branch, A.R.S., U. S. Dept. of Agriculture.
[3] Entomology Research Branch, A.R.S., U. S. Dept. of Agriculture.
[4] Iowa State College.

[5] Fertilizer attachment for applying fertilizer to corn at cultivating time. Manufactured by Ottawa Warner Corporation.
[6] A 3-foot plot-type grass seeder. Manufactured by International Harvester Company.
[7] Experimental 5-foot grass seeder. Manufactured by Century Engineering Corporation.
[8] Experimental corn planter attachments for applying granulated soil insecticides. Manufactured by E. S. Gandrud Company.
[9] A power take-off driven grass seeder. Manufactured by T. G. Tuttle & Sons & Company.
[10] Attapulgite materials were furnished by Attapulgus Minerals & Chemicals Corporation. Tobacco-base materials were furnished by Diamond Black Leaf Company. Bentonite materials were furnished by McConnon & Company. Vermiculite materials were furnished by Zonalite Company.

Table 2.—Physical properties of granulated insecticides.

Formulation			Emptying Angle of Repose[a] (Degrees)	Volume Weight[b] (Lbs./Ft.)	Other Properties
Carrier	Particle Size	Toxicant			
Attapulgite RVM	15/30	7½% DDT	38	41.8	
	30/40	7½% DDT	38	41.4	
	30/60	7½% DDT	37	39.2	
Attapulgite LVM	15/30	7½% DDT	40	45.1	
	30/40	7½% DDT	40	41.3	
	30/60	7½% DDT	39	42.0	
Tobacco	15/30	10% DDT	42	21.4	Special extracted
	20/40	10% DDT	42	18.6	Special extracted
	30/60	10% DDT	43	18.6	Special extracted
Attapulgite RVM	30/40	4% DDT	35	43.0	Adjunct added
	30/40	4% DDT	37	42.2	
	30/60	4% DDT	39	42.3	
Tobacco	30/60	4% DDT	41	30.3	Sterilized
	30/60	4% DDT	41	18.2	Special extracted
Bentonite (sodium)	30/40	4% DDT	37	73.0	Hydroscopic and easily dispersable in water
Perlite	20/40[c]	4% DDT	44	15.7	Heat expanded, hard particles resembling tiny bubbles of glass
Celite	30/60	4% DDT	60	27.2	Calcined aggregate, similar to diatomite
Vermiculite	20/60[c]	4% DDT	40	14.4	Heat expanded mica #4

[a] Angle at which material will stand as determined by filling a box approximately 5×4×3 inches, then removing one end and measuring the angle of the material.
[b] Determined with a Boerner weight per bushel apparatus.
[c] Approximate.

to provide an angle no less than 50° from vertical for all hopper sides, bottoms, and so forth, in order to avoid bridging.

Carriers were metered through the various machines to determine the effect that the speed of rotation of the metering mechanism had on the rate of discharge at a given setting. A typical set of curves from these tests are presented in figure 1. The expected normal operating speed for each machine was used. With the auger-fertilizer distributor the rate of discharge was directly proportional to the speed of rotation of the augers. This indicates that the auger-type metering mechanism could be used and, if ground driven, the rate of travel would have very little effect on the rate of application per acre. However, the rates obtained were too high for borer control work and this mechanism was not used in our field tests. The curve indicates that good performance could be obtained with this type of metering mechanism.

The rate of discharge obtained using the fluted-feed grass seeder was lower than desired and, because of this, the machine was excluded from the field tests. Performance tests using attapulgite RVM 30/60 showed the rate of discharge to be directly proportional to the speed of rotation of the flute. These results indicate that a fluted-feed metering mechanism would also work satisfactorily if the speed of rotation of the flute and the size

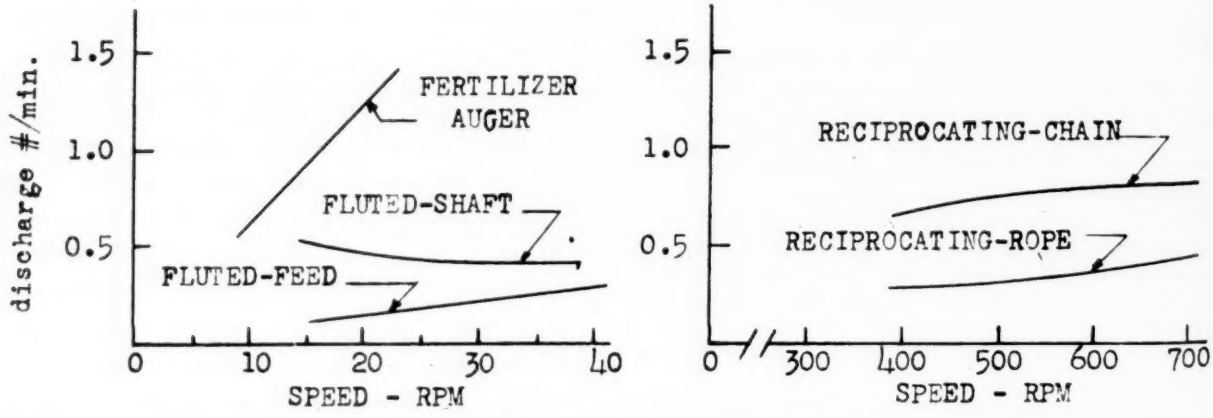

FIG. 1.—Speed discharge curves for applicators as indicated.

of the flute could be arranged to give the desired rate of discharge. A fluted-feed distributor was developed for field studies.

Results obtained with the fluted-shaft granular applicator showed that the rate of discharge decreased slightly as the rpm's of the shaft increased. In other words, at a given setting, this ground driven machine would apply a decreasing amount of material per acre as the speed of travel increased. The manufacturer developed a machine specifically for borer control that provided a shut-off mechanism and an adjustable discharge opening that eliminated these difficulties if a constant field speed was maintained. This new machine was used in the field studies.

The reciprocating-chain and rope seeders are normally operated from a tractor power take-off and the discharge speed curves are presented for the normal PTO operating speeds; namely, 400 to 700 r.p.m.'s. These two machines had similar speed-discharge curves. The rate of discharge increases with both machines only slightly as the r.p.m.'s increase from 400 to 700. For the chain seeder an adjustment was provided for each opening to insure uniform discharge from each. The rate of discharge from the rope seeder was regulated by lengthening or shortening the stroke and our tests indicate that there was considerable variation between the discharge of the openings. The reciprocating-chain seeder was used in the field tests.

Field Studies of First Brood Borers.—The reciprocating-chain seeder, the fluted-shaft applicator, and a modified grain drill fluted-feed mechanism were used in the first brood field experiments.

The reciprocating-chain seeder was discussed by Cox *et al.* (1956). For the 1955 work it was mounted on the boom hanger of a high clearance sprayer and driven by an auxilary engine through a flexible shaft. The discharge openings of this 5-foot experimental grass seeder are spaced 6 inches apart and as in previous years only the three openings over each row were used.

FIG. 2.—Rear view of the fluted-shaft granulated insecticide applicator that was used in the first generation studies. This commercially produced machine was designed specifically for corn borer control.

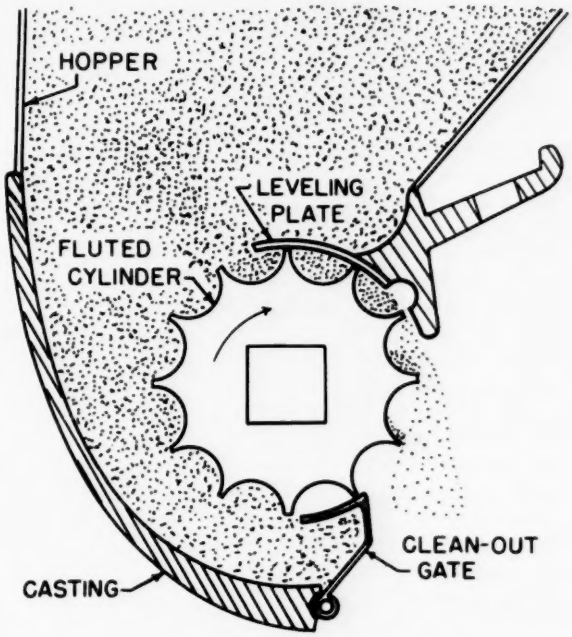

FIG. 3.—Cross-sectional view of the fluted-feed mechanism used to apply granulated insecticides. The leveling plate, direction of flute rotation and the flow of material are shown.

The commercially produced fluted-shaft applicator was designed to apply material to four rows at a time. The tilted flat plate beneath the three discharge openings above each row spaced 6 inches apart tended to even out the flow over 18 inches. This pull-type machine is ground driven and has an adjustable clearance of 3 to $5\frac{1}{2}$ feet. Figure 2 shows this machine in operation for first brood borer control.

The preliminary laboratory results indicated that the fluted-feed type applicator had good speed and rate of discharge relationships. In order to obtain the desired rates without excessive speeds the basic grain drill fluted-feed assembly was selected as a working unit. To avoid excessive leakage and a pulsating discharge the direction of rotation of the flute was reversed so that the material would be metered over the top of the flute. A leveling plate covering two flutes was inserted over the cylinder to control the amount of material carried in each flute and to prevent leakage between the top of the flute and the casting. To prevent the granules from spilling out the end of the flutes a washer was inserted between the flute and the cutoff. Figure 3 shows a cross-sectional view of the fluted-feed mechanism as used in the field tests. As shown in Figure 4 two of these units were attached to a high clearance machine and were driven by an auxiliary engine through a flexible shaft and a gear reduction unit.

These three machines and several different types of carriers were compared in a split-plot design experiment. Applications were made to the two center rows of the four row plots. Table 3 shows the results of this test. There was a high correlation between tunnel and larvae

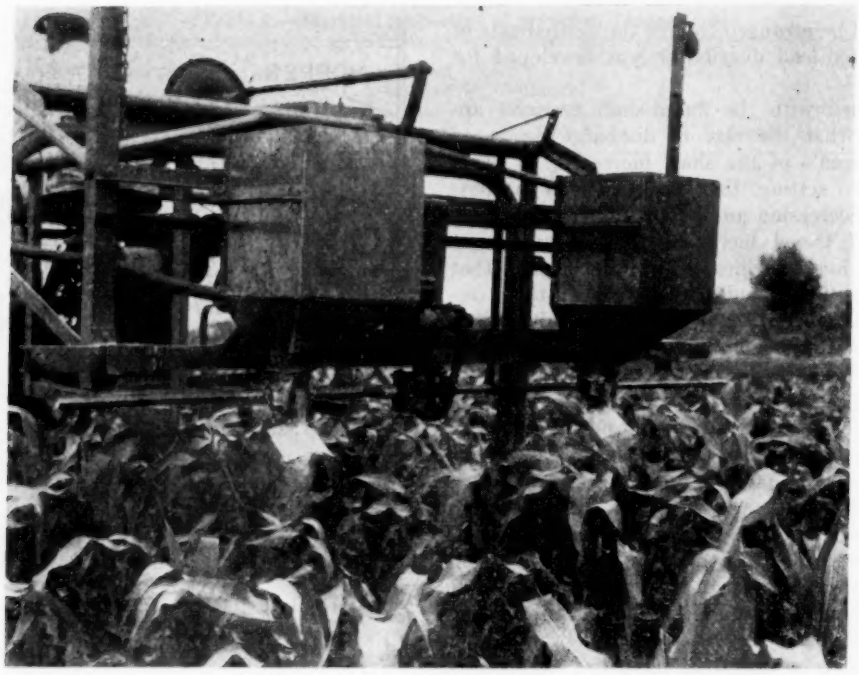

FIG. 4.—Rear view of fluted-feed applicator. As shown here a hopper with a fluted-feed was mounted on a high-clearance machine over each corn row. The spreader plate gave a swath width of 8 to 10 inches and was used for first generation studies.

counts and therefore only counts of the larvae are presented. The carriers and the control obtained using them was discussed by Cox *et al.* (1956). Differences in control among the machines as measured by counts of larvae were not significant at the 5% level. There were some interesting trends in these data. With the bentonite carrier, which has the highest volume weight, the best control was obtained with the fluted-shaft applicator. With perlite, Celite, and vermiculite, some of the lighter materials, the reciprocating-chain gave the best control. All machines gave approximately the same control when the attapulgite carrier was used, however, the fluted-feed gave slightly poorer control than the others. The fluted-feed and reciprocating-chain gave better control than the fluted-shaft when tobacco was used as a carrier. Yield differences among machines were significant at the

Table 3.—Surviving larvae, DDT residues, and yields obtained by applying different DDT granulated formulations with three types of application equipment.

	Carrier[a]									
	Attapulgite RVM			Tobacco						
	30/40 with adjunct	30/40	30/60	30/60 Steril-ized	30/60 Special Extracted	Bentonite 30/40	Perlite approx. 20/40	Celite 30/60	Vermic-ulite approx. 20/40	Average
	Surviving larvae per 100 plants[b]									
Fluted-feed	35.0	57.5	33.7	41.2	32.5	58.7	66.2	82.5	95.0	55.8
Reciprocating-chain	35.0	30.0	23.7	36.2	41.2	67.5	41.2	58.7	46.2	42.2
Fluted-shaft	27.5	30.0	38.7	50.0	58.7	25.0	57.5	61.2	106.2	50.5
Untreated shaft										174.0
	P.p.m. DDT in corn whorl[b]									
Fluted-feed	57.3	60.9	90.2	38.6	63.1	90.1	36.4	50.7	18.8	58.2
Reciprocating-chain	54.3	48.1	46.6	25.4	36.7	31.1	31.6	60.2	35.1	41.0
Fluted-shaft	69.0	37.8	50.9	52.0	20.9	34.5	23.6	68.2	11.4	40.9
	Yield in bushels per acre[b]									
Fluted-feed	62.4	53.0	52.1	49.9	56.1	66.4	46.1	60.9	58.5	56.2
Reciprocating-chain	59.8	61.5	68.8	59.1	60.5	62.5	63.1	61.2	58.2	61.6
Fluted-shaft	56.9	53.1	55.3	61.2	58.3	65.0	54.4	54.1	51.6	56.6
Untreated check										51.5

[a] All formulations contained 4% DDT and were applied at the rate of 20 pounds per acre on June 21 when 95% of the plants were showing leaf feeding.
[b] Each number represents the average of four replications.

5% level and the mean yield for all carriers was highest when the reciprocating-chain was used. However, the correlation coefficient between yields and counts of larvae was very low indicating that differences in yield were not necessarily caused by the borer infestation. Residue data show that the reciprocating-chain gave lower residues for all carriers except vermiculite and in general the fluted-feed gave the highest residue. The fluted-feed had approximately an 8-inch width of spread of material over the row as compared to 18-inch spread with the other machines and this may be the reason why the residues are higher. There was very little correlation between residues and larvae counts and between residues and yields.

A split-split-plot design experiment was used to compare machines, carriers, and particle sizes. The three machines mentioned above were also used in this experiment. Attapulgite RVM, attapulgite LVM, and tobacco base materials were the carriers used. Three particle sizes: namely, 15/30, 30/40, and 30/60 were used. However, the tobacco base material was not formulated in a 30/40 size and therefore a 20/40 size particle was substituted. Three-fourths of a pound of actual DDT was applied for each treatment. Since the attapulgite carriers were available as 7.5% formulations and the tobacco base as a 10% formulation it was necessary to apply 10 and 7.5 pounds of granules per acre, respectively. These treatments were made on June 20 when 90 to 95% of the plants showed leaf feeding. Table 4 shows the results of this test.

All treatments showed a significant reduction in borers over the check plots. In the overall analysis of the data on the larvae there were no differences at the 5% level among the machines. There was a significant application equipment insecticide carrier interaction. When the reciprocating-chain seeder was used to apply attapulgite RVM poorer control was obtained than when this same material was applied with the fluted-shaft applicator or with the fluted-feed applicator. When attapulgite LVM was used the fluted-shaft applicator gave poorer control than the reciprocating-chain or the fluted-feed.

The data from the experiment on machines, carriers, and particle sizes show that the machine differences were not significant at the 5% level. For the 15/30 and 30/40 particle sizes of the attapulgite RVM material the reciprocating-chain gave poorer control than the fluted-shaft or the fluted-feed. However, when the 30/60 particle size was used the fluted-shaft gave poorer control than the other two. The fluted-shaft showed poorer control than the other two machines for all sizes of the attapulgite LVM material. With the exception of the 15/30 particle size tobacco, which showed the poorest control when applied with the fluted-shaft, approximately equal control was obtained with all machines with the tobacco carriers. The larvae tunnel correlation was evident and only the data on larvae are presented. Yield data were obtained for attapulgite RVM materials and indicate that lower yields were obtained when the reciprocating-chain was used. In this experiment the highest amount of residue was found where the fluted-feed was used. With the attapulgite RVM materials the reciprocating-chain gave the smallest amount of residue while with the attapulgite LVM materials the fluted-shaft gave the smallest amount of residue.

Field Studies of Second Brood Borers.—Two additional machines were developed for our second brood tests. Internal run seeding mechanisms were mounted on hoppers that were in turn attached to the high clearance machine similar to the fluted-feed machine already discussed. Figure 5 shows this machine with a spreader plate.

An auger feed was mounted in the bottom of a conventional duster hopper to meter the material into a separating fan where it was equally divided and carried in an air stream to a cyclone separator mounted on the boom directly over each row. The air was relieved in the

Table 4.—Surviving larvae, DDT residue, and yields from applying different DDT granulated formulations of different sizes with three types of application equipment.

	Carrier[a]									
	Attapulgite RVM			Attapulgite LVM			Tobacco			
Applicator	15/30	30/40	30/60	15/30	30/40	30/60	15/30	20/40	30/60	Average
	Surviving larvae per 100 plants[b]									
Fluted-feed	57.5	43.8	42.5	122.5	86.3	82.5	113.8	98.8	98.8	82.9
Reciprocating-chain	106.3	170.0	61.3	112.5	91.3	103.8	108.8	108.8	92.5	106.1
Fluted-shaft	78.8	46.3	76.3	170.0	182.5	175.0	162.5	105.0	117.5	123.8
Untreated check										292.5
	P.p.m. DDT in corn whorl[b]									
Fluted-feed	85.9	112.0	105.1	77.5	139.7	83.6	97.6	63.9	67.5	92.5
Reciprocating-chain	23.6	17.6	42.9	53.9	66.6	39.9	54.0	61.8	41.1	44.4
Fluted-shaft	42.8	67.2	67.5	18.6	24.1	16.9	82.3	64.9	44.1	47.6
	Yield in bushels per acre[b]									
Fluted-feed	53.7	65.0	56.8							61.8
Reciprocating-chain	40.2	47.0	44.1							43.8
Fluted-shaft	54.1	51.9	63.0							56.3
Untreated check										43.7

[a] RVM and LVM materials contained 7½% DDT and were applied at the rate of 10 pounds per acre. Tobacco materials contained 10% DDT and were applied at the rate of 7½ pounds per acre.

[b] Each number represents the average of four replications.

FIG. 5.—Close-up view of the internal-run mechanism showing the corrugated spreader plate used to obtain a spread of 16 to 18 inches. This unit was attached to a hopper in the same manner as the fluted-feed shown in figure 4.

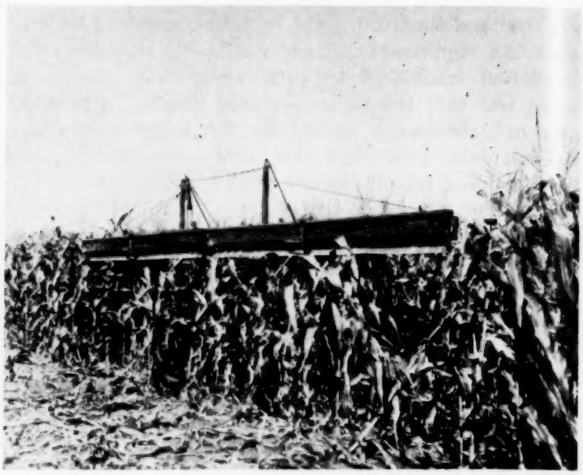

FIG. 7.—Rear view of high-clearance fluted-shaft applicator used to apply granulated insecticides for second generation borer control. Note the flow of material over the two center rows which were used in the experiment.

cyclone separator and the material was discharged in a circular motion onto the corn plants. This type of machine avoids heavy loads on the boom-hanger and facilitates loading and cleaning. Figure 6 shows this machine during second brood applications.

The same spreader plate shown in figure 5 was used on the fluted-feed applicator for second generation tests. The machine was essentially the same as the fluted-feed used for first brood studies.

The manufacturer of the fluted-shaft applicator mounted two units like the one used for first brood tests onto a high clearance spray-rig. This high clearance machine was used for the second brood tests. Figure 7 shows this machine in the field.

The reciprocating-chain was the same as used for first brood tests.

A randomized block type experiment was set up to test five machines using attapulgite RVM 30/60 with a 7.5% DDT formulation. Twenty pounds of material was applied per acre for all treatments. Table 5 shows the results obtained.

Results from first brood studies indicated that the width of spread influenced the control of the borers. To

FIG. 6.—Rear view of experimental auger-cyclone mechanism developed to apply granulated materials. One cyclone separator was mounted over each row of corn.

Table 5.—Surviving larvae and DDT residue from applications of 7.5% DDT formulation of attapulgite RVM 30/60 granules at the rate of 20 pounds per acre with five types of applicators.

Applicator	Larvae per 100 Plants					P.p.m. DDT on Stalk and Leaf Axils from Ear to Top of Plant				
	Replicate					Replicate				
	A	B	C	D	Average	A	B	C	D	Average
Fluted-feed	190	80	10	190	118	6.1	8.8	9.1	12.0	9.0
Reciprocating-chain	90	90	40	30	63	12.9	6.1	6.8	6.7	8.1
Fluted-shaft	30	60	20	30	35	14.5	9.5	16.2	14.9	13.8
Internal-run	80	160	130	90	115	2.3	10.2	6.8	12.5	8.0
Auger-cyclone	90	40	60	60	63	6.0	8.1	12.9	8.6	8.9
Untreated	180	220	70	140	153					

study this further a randomized block experiment was set up whereby the material was spread in 6-inch, 12-inch, and 18-inch bands. The fluted-feed applicator was used for these studies. As shown in figure 5 a piece of sheet metal fabricated into a fan like structure was mounted below the flute. The width of spread could be changed by raising and lowering the fan spreader. The results of this test are shown in table 6.

As in the first brood experiments all machines showed a significant reduction in borers. The analysis of the data from these two experiments showed no differences at 5% level among the machines or swath widths. The extended egg deposition period and the spotty infestation made good control extremely difficult. The large variation of control within any treatment makes it necessary to have very large differences for significance. The fluted-feed and internal-run show poorer control than the other machines and the fluted-shaft showed the best control. Residues were low and there was very little correlation between residues and borer counts. There is, however, an indication in the swath width experiment that the wider swath width gave better control.

SUMMARY.—Experiments were conducted with several metering mechanisms for the application of granulated formulations of insecticides for control of the European corn borer.

Five machines and seven types of granulated carriers sized from 30/60 to 8/20 were tested in the laboratory. The angle of repose for all of these materials was very similar and varied from 35° to 60°. If the flow of material depends on gravity, equipment should be designed to provide an angle of no less than 50° with the vertical. Volume weights varied from 14.3 for vermiculite to 73 for bentonite. Using these two extremes as an example and assuming a fixed percentage formulation of insecticide the discharge per minute would be six times greater for the vermiculite than for the bentonite. To use these carriers application equipment should be provided with adjustable rates of discharge to take care of this variation. The physical breakdown of granules that were metered through the various machines was not serious. The rate of discharge in pounds per minute was determined for the normal operating speeds of the various metering mechanisms. The fertilizer auger and the fluted-feed gave rates of discharge that varied directly with the speed of rotation of the metering mechanism. The rate of discharge from the fluted-shaft decreased slightly as the speed of rotation of the shaft increased. The reciprocating-chain and the reciprocating-rope showed discharge rates that increased slightly as the speed of reciprocation increased. The auger-type or the fluted-feed type would be more satisfactory for ground driven applicators if the speed of travel in the field could not be controlled. All of these mechanisms showed good speed discharge relationships when the speed was held constant.

The fluted-feed, the reciprocating-chain, and the fluted-shaft machines showed significant reductions in corn borer larvae for all carriers and all sizes over the untreated checks in the first brood studies. Yields were greater on the treated plots than on the untreated. Differences in surviving corn borer larvae among the machines for the different carriers and different sizes of carriers were not significant at the 5% level. Yield differences were significant in one experiment. The amount of DDT residue on the plants was similar for all machines. However, the fluted-feed showed higher amounts than the other two machines. The data from

Table 6.—Surviving larvae and DDT residue from applying attapulgite RVM 30/60 with 7.5% DDT at the rate of 20 pounds per acre using three different widths of spread.

Width of Spread (Inches)	Larvae per 100 Plants					P.p.m. on Stalk and Leaf Axils from Ear to Top of Plant				
	Replicate					Replicate				
	A	B	C	D	Average	A	B	C	D	Average
6	50	90	80	90	78	5.3	12.2	6.8	6.8	7.8
12	160	20	30	70	70	4.2	6.8	4.7	5.2	5.2
18	40	40	60	50	48	16.0	6.6	16.5	6.3	11.4
Untreated	140	150	60	290	160					

these studies indicate that all three machines could be used to apply granulated insecticides successfully to control first generation borers.

All five metering mechanisms were used to control borers for second brood studies and all showed reductions in borers when compared with the untreated checks. There were no apparent differences among the machines tested. Residue analysis of the stalks and axils shows practically no differences among the machines. The 12- and 18-inch width of spread gave slightly better borer control than 6-inch width.

Some of the general requirements for an applicator of granulated insecticides to control European corn borers in field corn are as follows:

1. A positive metering mechanism that will handle materials having volume weights of 10 to 75 pounds per cubic foot.
2. For first brood control ground clearance of 3 to 5½ feet for second brood control clearance of 8 to 12 feet.
3. If ground driven, machines should have a discharge rate that is directly proportional to the speed of the metering mechanism or an adjustable discharge opening and a means for maintaining constant field speeds.
4. If the metering device is not ground driven the discharge rate should be constant for the normal operating speed at a given opening.
5. Spreaders should be used over each row to obtain a width of spread of 12 to 18 inches.
6. A positive shut-off device should be used to prevent excessive waste.
7. Physical breakdown of particles should be held at a minimum.
8. Provisions should be made to make loading, unloading, cleaning and calibration of the equipment as easy as possible.

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Residues on Plants Treated with DDT Granules and Emulsions for European Corn Borer Control[1]

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Studies of granulated insecticides for control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) have been conducted on the Iowa State College Experimental Farm since 1953. Fahey *et al.* (1953) reported studies of the distribution of DDT residues on corn plants and showed that more than 90% of the total residue was on the leaves. Cox *et al.* (1956), reporting work done in 1954, confirmed these observations and showed that when granulated insecticides were applied to corn plants the leaves did not carry any greater residues than the stalk and axil. In 1955 the project for control of European corn borer with granulated insecticides was expanded. In the study of residues special emphasis was given to comparison of the distribution of residues on plants treated with granulated and emulsion formulations of DDT. Observations on the control of corn borer with granulated insecticides by Cox *et al.* (1956) emphasized the comparison of insecticides, formulations, and rates and time of application, while Lovely *et al.* (1956) studied applicators for granulated insecticides.

SAMPLING FOR RESIDUE ANALYSES.—The plots employed for study of insecticide residues were four rows wide, and the two center rows were used for sampling. Details of plot arrangement, timing and insecticide applicators used are described by Cox *et al.* and Lovely *et al.* in papers referred to above. Samples for residue analysis consisted of plants collected at random from each of four replicated plots. The plants were divided into four parts—whorl tips, whorl, leaves, and stalk and axils—for study of first-brood treatments, and into three parts—leaves, husk, and stalk and axil—for study of second-brood treatments (figure 1). For study of the distribution of residues on the plant, analogous portions of 10 dissected plants from each replicate were pooled for analysis. In the comparison of insecticide formulations only the plant whorls were analyzed. The residue analyses were made by the Stiff-Castillo colorimetric method (Fahey & Rusk 1951).

DISTRIBUTION OF INSECTICIDES.—*First brood.*—Insecticides for control of first-brood borers are applied while the plants are in the whorl stage. At this time the plant is small, about 40 inches high, and in the center is a well-defined whorl of leaves. The tips of these leaves form a natural funnel into the whorl. The side leaves of the plant are at acute angles to the stalk and tend to form troughs or slides to the leaf axils.

The results of the analyses of samples following first-

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First brood Second brood

FIG. 1.—Diagrammatic dissection of corn plant treated for control of corn borers.

brood applications of DDT granules and emulsion are given in table 1. Where the emulsion was applied, the tips of the leaves retained more residues than the whorl or stalk and axils. This was due to the fact that sprays struck the leaves first and did not penetrate to the whorls and axils. Where granules were applied, the whorl tips and leaves retained very little residue because there was no liquid to hold the particles to the leaf surface. The particles fell and/or rolled into the leaf axils and into the plant whorl. Fahey *et al.* (1953) have demonstrated that no residue is left on field corn at ensiling time by emulsion sprays applied for control of first-brood borers, if the final spray is applied prior to July 5.

Second brood.—Insecticides for control of second-brood borers may be applied at any time between July 15 and August 15, depending upon insect development. Usually two applications are made 5 days apart. By this time the corn plant has a structure different from the time of first brood applications. The whorl has disappeared, the tassel and ears have developed, and the leaves have lost most of their pubescence and become hard and waxy.

The results of analyses of corn treated with DDT granules or emulsion for control of second brood borers are given in table 2. As with the first brood, the plants treated with an emulsion retained a heavier residue on the leaves than those treated with granules. The granules

Table 1.—Distribution of DDT residues (parts per million) on corn plants treated for control of first-brood corn borers.

Dates Applied (June)	Dates Sampled (June)	Granules				Emulsion			
		Tips of Whorl Leaves	Whorl	Leaves	Stalk and Axils	Tips of Whorl Leaves	Whorl	Leaves	Stalk and Axils
One application:									
2	14	7.1	3.8	2.4	2.1	37.8	1.5	96.2	0.3
9	19	1.9	1.5	1.7	1.4	20.2	0.6	77.7	0.9
14	24	1.8	3.5	1.3	2.4	23.1	0.9	67.4	0.3
Two applications:									
2, 9	14	11.1	17.4	3.1	5.4	216.4	14.2	219.4	2.7
9, 14	19	9.5	7.5	2.8	7.1	161.4	2.4	203.6	1.4
14, 19	24	9.0	22.9	3.8	6.8	167.3	5.0	186.3	1.5
Three applications:									
2, 9, 14	14	11.7	60.0	1.4	12.9	516.0	31.9	371.7	2.9
9, 14, 19	19	11.5	76.9	4.8	14.5	494.0	36.5	357.2	3.6
14, 19, 24	24	25.1	143.4	9.0	25.2	585.2	30.3	346.3	3.1

Table 2.—Distribution of DDT residues (parts per million) on corn plants treated for control of second-brood corn borers

Dates Applied	Date Sampled	Granules			Emulsion		
		Husks	Leaves	Stalk and Axils	Husks	Leaves	Stalk and Axils
One application:							
July 25	July 30	0.8	4.8	10.1	1.2	46.5	7.6
August 4	August 9	2.1	6.0	20.0	3.9	65.3	7.5
14	19	3.8	13.9	29.2	14.9	184.2	17.8
Two applications:							
July 25, 30	July 30	2.7	5.0	25.4	14.5	262.0	22.5
August 4, 9	August 9	3.2	10.7	26.1	24.5	337.7	36.1
14, 19	19	6.1	12.6	40.5	36.5	407.9	57.9

generally left more residue on the stalk and leaf axil, but the difference was not as great as in applications against the first brood. From both formulations the residues on the stalk and axil were generally greater than from applications for control of the first-brood because of the more open plant structure at this time.

At ear harvest samples of corn stover were collected from plots that had received two applications of DDT granules or emulsion. For analysis the plants were divided into two parts—leaves and husks, and stalk and axils. The results of these analyses are given in table 3. Animals foraging in corn stover generally feed on the leaves and husks. Analyses show this portion of the plants treated with granules contained on an average only 14 p.p.m. of DDT as compared with 136.4 p.p.m. on the plants treated with the emulsion.

EFFECT OF DIFFERENT CARRIERS.—A number of materials have been proposed for use as carriers for granulated formulations. An experiment was arranged to compare granules prepared with nine carriers. Each granulated formulation was applied with three machines—a fluted-feed seeder, a chain-type seeder, and a fluted-shaft seeder, described by Lovely *et al.* 1956. The results of analyses of the whorl section of samples from these plots are given in table 4. Each figure in this comparison represents the average residue from 120 corn plant whorls—i.e., 10 plants from each of four replicates treated with three machines. The attapulgite 30/60 mesh and 30/40 mesh with adjunct and Celite left the greatest residues.

In a second experiment with carriers for granulated insecticides the effect of particle size (mesh) was studied. Three carriers were used—RVM and LVM attapulgite and tobacco stems—each in 15/30, 30/40 and 30/60 mesh. As in the preceding experiment, each formulation was applied with three machines to plots replicated four times. The data, given in table 5, fail to show any consistent difference in residues in relation to particle size.

Table 3.—DDT residues (parts per million) on corn stover following two applications for control of second-brood corn borers.

Dates of Application	Granules		Emulsion	
	Leaves and Husks	Stalk and Axils	Leaves and Husks	Stalk and Axils
July 25, 30	9.5	5.9	134.7	10.8
August 4, 9	17.0	9.7	131.4	12.7
14, 19	15.6	11.6	143.2	10.5

Table 4.—Comparison of DDT granules impregnated on different carriers.

Carrier Used	Mesh Size	P.P.M. of DDT in Plant Whorl
Attapulgite, RVM-AA	30/60	62.6
Attapulgite, RVM-AA with adjunct	30/40	60.2
Celite, diatomite, calcined	30/60	59.7
Bentonite, granulated	30/40	51.9
Attapulgite, RVM-AA	30/40	48.9
Tobacco base, extracted	30/60	40.2
Tobacco base, sterilized	30/60	38.7
Perlite, volcanic glass	20/40[a]	30.5
Vermiculite, expanded mica	20/40[a]	21.8

[a] Approximate size.

RESIDUES FROM GRANULES APPLIED WITH DIFFERENT MACHINES.—In the study of machinery for application of granules five types of machines were used. The plots were treated for second-brood borer control. Residue analyses of plant-part samples (representing 40 corn plants) failed to show any appreciable difference between machines.

In another experiment three machines—fluted feed, a

Table 5.—Study of effect of size of granules on residue deposit.

Carrier	Mesh Size	P.P.M. of DDT in Plant Whorl
Attapulgite		
LVM	15/30	50.0
	30/40	76.8
	30/60	47.6
RVM	15/30	50.8
	30/40	65.6
	30/60	71.8
Tobacco base	15/30	77.9
	30/40	63.5
	30/60	50.9
Average, All carriers	15/30	59.6
	30/40	68.6
	30/60	56.8

Table 6.—Parts per million of DDT deposited in the plant whorl when applied with different types of machines.

MACHINE	AVERAGE OF 9 CARRIERS	AVERAGE OF 3 CARRIERS OF 3 MESH SIZES
Fluted-feed	56.2	92.5
Chain-type	41.0	44.8
Fluted-shaft	44.8	47.6

chain-type, and a fluted-shaft seeder—were employed on plots treated with nine different carriers and on nine plots treated with three carriers of three mesh sizes. Samples of 10 plant whorls were collected from four replicates of each treatment; thus the data obtained compare the analyses of 360 plant whorls treated with each machine. Table 6 shows that the fluted-feed seeder left a heavier residue in the plant whorl than either the chain-type or fluted-shaft seeder.

SUMMARY.—The distribution of DDT residues on corn plants from the application of an emulsion spray and granulated formulation was studied at Ankeny, Iowa, in 1955. The emulsion deposited greater residues on corn leaves than did granules, but the granules deposited equal or greater residues in the plant whorls and the leaf axils.

Granulated formulations prepared with attapulgite or Celite gave greater deposits than formulations prepared with other carriers.

An experiment designed to compare applicators, employing single treatments replicated four times, failed to show significant differences between applicators. A comparison of 3 machines, in which 2 samples of 36 ten-plant replicates treated with each machine were analyzed, showed the fluted-feed applicator to be superior to the chain-type or fluted-shaft seeder.

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Effect of Acetone on Toxicity in Pyrethrum and Allethrin Space Sprays[1]

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When the relative toxicity of materials applied to insects by means of a settling mist in the Campbell turntable (Campbell & Sullivan 1938) is evaluated, the actual dosage received by the insects is not measured. Conditions are so controlled that the amount of spray settling on the insects is the same in each test in any given series and mortalities are related to the concentration of the original spray. In the last decade the turntable has been found to be a useful apparatus in such evaluations and many comparisons of the toxicity of insecticides in kerosene sprays have been made with it. However, it has been necessary, especially in studies on joint action, to test many substances not sufficiently soluble in kerosene alone at the concentrations desired for the stock solutions. An auxiliary solvent, acetone, has often been used. However, it has been apparent, when proper standards of comparison were included in the testing and when concentrations of the sprays were stated in terms of the total volume, that sprays containing acetone, even when in small amount, cause appreciably higher mortalities than sprays containing the same amount of the insecticide in oil alone. Acetone when applied alone by this procedure did not cause mortality. As it seemed probable that an auxiliary solvent, especially one so volatile as acetone, would affect physical conditions in the test cylinder, such as particle size and concentration, care was taken in critical comparisons to maintain the concentration of that solvent the same in all sprays in the study. It was believed that under such uniform conditions relative toxicities found by the regular procedure of estimation would be valid so long as no material was precipitated.

To gain information on the effect of acetone, and in particular to find, if possible, a method of correlating relative toxicities estimated in sprays of different acetone content, a study was made in 1945 in which pyrethrum extractive was used in refined kerosene, alone and mixed with acetone in several proportions. The mortality results have not been published but have been frequently used in planning and other preliminary work in this laboratory. However, recently a study measuring the joint action of certain compounds with pyrethrins or allethrin was reported (Gersdorff *et al.* 1956) which restricted the standards to kerosene sprays only whereas some of the mixed sprays contained acetone. To put the evaluations of relative toxicity for these sprays on a sim-

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ple comparable basis by allowing for the increase in toxicity due to acetone, it was necessary to make a statistical analysis of the unreported data. This paper reports the results of this analysis.

METHODS.—Sprays were prepared at concentrations of 0.50, 1.00, and 2.00 mg. of pyrethrins per milliliter in refined kerosene (Deobase) only and also in acetone-kerosene mixtures containing 25, 50, and 75% of acetone by volume. Sprays at the same concentrations were prepared with acetone as the sole diluent of the stock supply of pyrethrins. However, 100% pyrethrins, or even 20% concentrates, not being available, the stock solution of pyrethrins was at the concentration of the common commercial concentrate, 20 mg. per milliliter of kerosene. Therefore, 2.5, 5, and 10% respectively, of kerosene were present in these three sprays. In the sample of the concentrate used 55% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method.

With these sprays knockdown and mortality were determined in replicated tests by the turntable method with the house fly, *Musca domestica* L., as the test insect. Approximately 160 flies, reared by standard procedure and averaging 2 days in age, were used in each test. All sprays were tested in duplicate simultaneously on each of six populations of flies.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952).

KNOCKDOWN AND MORTALITY RESULTS.—Knockdown in 25 minutes was complete for all sprays. The data for mortality in 1 day are summarized in table 1. In addition to the actual concentrations of the sprays, concentrations were calculated on the basis of the kerosene content of the mixed solvent. Except with the high-acetone sprays there was a marked rise in mortality with increase in acetone content at all three actual concentrations. The relationship between mortality and concentration stated on the basis of kerosene content only will be more readily seen after the statistical treatment of the data.

EVALUATION OF RELATIVE TOXICITY.—To put the relationships of the different types of sprays on a precise quantitative basis, provisional regression lines were fitted graphically to the data. Since it was apparent that the lines for all but the high-acetone type were approximately parallel, new provisional lines for these types were fitted to a common slope and the procedure for generalization was used. In the subsequent analysis of chi-squares there was no evidence of any conflict with the hypothesis that the data for the four types could be fitted with parallel lines. No heterogeneity factors were required.

The weighted regression equations were computed. These final equations, showing the regression of mortality, expressed in probits, on concentration in milligrams of pyrethrins per deciliter, based on total volume and expressed as logarithms, together with the standard errors of the regression coefficients (b), are given below.

Per cent acetone		*Standard error of b*
0	$Y=2.4924\,X-0.5266$	0.041
25	$Y=2.4924\,X-0.0812$	.041
50	$Y=2.4924\,X+0.3986$	.041
75	$Y=2.4924\,X+0.8994$	.041
100 (nearly)	$Y=3.9594\,X-1.8106$	.114

From these equations median lethal concentrations (LC-50's) were estimated and toxicities relative to pyrethrins in kerosene only were calculated as the inverse ratios of the relevant pair of estimations. Since the relative toxicity of pyrethrins in the high-acetone mixture cannot be expressed by a single figure, even approximately, because the lines are too divergent, ratios were also estimated at the 5% and 95% mortality levels for

Table 1.—Relative toxicity of pyrethrins in acetone-kerosene sprays compared with that in kerosene only.

ACETONE (PER CENT BY VOLUME)	PYRETHRINS (MG. PER DL.)		MEAN PER CENT MORTALITY (12 REPLICATES)	LC-50[a] (MG. PER DL.)		RELATIVE TOXICITY	
	Actual	Calculated in Kerosene Only		Actual	In Kerosene Only	Actual	In Kerosene Only
0	200	200	59.8	165.0 ±3.0	165	1.0	1.0
	100	100	28.3	(36.09)[b]			
	50	50	9.2	(754.0)[c]			
25	200	267	73.9	109.3 ±1.8	146	1.51±0.037	1.13
	100	133	46.3				
	50	66.7	20.1				
50	200	400	86.6	70.17±1.21	140	2.35±0.059	1.18
	100	200	64.9				
	50	100	36.4				
75	200	800	95.6	44.18±0.92	177	3.73±0.103	0.93
	100	400	79.1				
	50	200	56.5				
90	200	2000	98.9	52.59±0.80	—	3.14±0.074	—
95.5	100	2000	86.7	(20.17)[b]	—	(1.79)[b]	—
97.5	50	2000	46.6	(136.6)[c]	—	(5.52)[c]	—

[a] Except as indicated.
[b] Concentration and ratio at 5% mortality level.
[c] Concentration and ratio at 95% mortality level.

this mixture. The estimations together with the standard errors needed are also given in table 1.

DISCUSSION.—The course of action of the high-acetone sprays is quite different from that of the other types of spray. Nothing is known regarding the possible effects of the small amounts of kerosene in the sprays; moreover, a hypothesis regarding the proportion of the two solvents in the droplets obtained within the chamber cannot be made with this type of mixture. Therefore, the results obtained with these sprays will be omitted from the following discussion.

Study of the regression lines, LC-50's, or ratios of toxicity for the four other types of spray shows that relative toxicity varies geometrically as acetone content in the original spray varies arithmetically. The ratios of toxicity corresponding to the successive 25% increments in acetone are 1.51, 1.55, and 1.59, with a mean of 1.55. For smaller increments the ratio may be interpolated or estimated directly from the equation showing the relationship of the two variables. This equation is

$$R = e^{1.758pA}$$

in which R is the ratio of toxicity relative to pyrethrins in kerosene and pA is the proportion of acetone in the mixed solvent between 0 and 75%. In the use of such estimated ratios in a recent joint-action study (Gersdorff *et al.* 1956), the relative toxicities for sprays containing 0, 2.5, 5, 10, 15, and 20% of acetone were estimated to be, respectively, 1.0, 1.044, 1.092, 1.192, 1.302, and 1.421.

A similar study of acetone-kerosene mixtures with allethrin as the toxicant has not been made. However, because information on this problem was desired, additional sprays have been included from time to time in other studies to supply pertinent data. From the latter there have been calculated 12 approximate ratios showing the increase in toxicity accompanying a 25% increment in acetone content (for the range of 0 to 25 as well as 25 to 50%)—1.18, 1.27, 1.28, 1.32, 1.44, 1.44, 1.52, 1.52 1.76, 1.84, 1.85, and 1.88, with an unweighted mean of 1.53. Thus, it appears that the effect of acetone in allethrin sprays does not differ much, if at all, from that in pyrethrum sprays.

One cause of a higher mortality with the spray containing acetone than with the spray of the same concentration of pyrethrins in kerosene alone might be a material reduction in the size of the droplets. Smaller droplets could result in a delay in settling during the 10-minute exposure period and consequently in a smaller deposit on the flies. However, there would also be a smaller deposit on the slide before its withdrawal and consequently an increase in the amount of spray held in the cylinder. It is impossible to estimate what would be the resultant of these two effects in this procedure, in which the maximum amount of mist obtainable under the existing conditions is sought by spraying a large amount in a small space and the spraying is accompanied by high turbulence in the cylinder. Exposure in an atmosphere containing acetone would be a further complication. Nevertheless, the conditions approach those that would exist if the amount of spray received by the flies remained the same but the concentrations were increased by volatilization of practically all the acetone, the pyrethrins remaining in solution in the kerosene. When the concentrations are based on kerosene content, the equations for the mixtures containing 25, 50, and 75% of acetone become very nearly the same as the equation for pyrethrins in kerosene alone, the constants becoming 0.393, 0.352, and 0.601 as compared with 0.527. As shown in table 1, the new LC-50's approximate that of pyrethrins in kerosene alone, and therefore the new ratios of toxicity approximate unity. These relationships indicate that the conditions within the cylinder approach those that would exist if the acetone volatilized completely. Actually, it cannot be as simple as this; in fact, the causing of less than complete mortality by high-acetone sprays shows that these at least were substantially diluted with acetone. But the evidence is strong that valid determinations of the relative toxicity may therefore be obtained with acetonic mixtures provided the materials remain dissolved in the kerosene and the effect of the acetone is allowed for. However, there are serious doubts of meaningful results from such mixtures in the comparison of toxicity by a settling-mist method if an ingredient is not completely soluble at the concentration in kerosene. With the solutions the effects as measured with the original concentrations of the sprays are real, because the dosages are increased and an action that may at first appear to be synergistic is explainable on simple physical grounds.

SUMMARY.—Tests were made to study the effect of acetone in pyrethrum-kerosene sprays on toxicity to house flies, *Musca domestica* L., when applied on the Campbell turntable.

When calculated by the usual procedure, pyrethrins in mixtures containing 25, 50, and 75% of acetone were, respectively, 1.51, 2.35, and 3.73 as toxic as pyrethrins in kerosene alone throughout the course of action. The action in the sprays containing 90% or more of acetone was quite different.

The ratio of toxicity (R) of pyrethrins in the original mixed solvent to that in kerosene alone varied geometrically as the proportion of acetone (pA) varied arithmetically between 0 and 75%, the ratio corresponding to a 25% increment in acetone being 1.55. The equation showing this relationship is $R = e^{1.758\ pA}$. The effect of acetone in allethrin sprays was about the same.

The effect is apparent because of the method of comparing concentrations based on the original total volume and is explainable on purely physical grounds, most of the acetone volatilizing with a consequent increase in the dosage of insecticide applied to the insects. The relative toxicities of the toxicants are not affected, but it is necessary for valid comparisons to use standards with the same proportion of acetone to kerosene or to allow for differences in proportion.

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The Effect of Pink Bollworm Infestations on Yield and Quality of Cotton[1]

J. R. Brazzel[2] and J. C. Gaines[3]

The pink bollworm, *Pectinophora gossypiella* (Saund.), has been a potential pest of cotton in the Southwestern United States for approximately 40 years. In some areas, this insect has been credited with reducing the value of the cotton crop up to 30%. It is generally agreed that most of this loss is caused by reduced quality of the cotton (Anonymous 1954). Hitherto, the experiments conducted to evaluate the yield and quality losses caused by pink bollworm have been complicated by the presence of other cotton pests. The results of such investigations reflected a composite of the damage of all the destructive insects present in the cotton. The experiment herein discussed was designed to reflect an accurate measurement of yield and quality losses resulting from various levels of pink bollworm infestations in the absence of all other cotton pests in the test plots.

Methods.—A randomized block design was used with each treatment replicated four times. The plots were 1/200 acre in size and each was covered with a 6 ft. ×6 ft. × 36 ft. plastic screen cage. The screen was 14×18 mesh and excluded all other pests except mites and aphids, which were controlled by two foliage applications of schradan.

Three levels of pink bollworm infestations and an insect-free check were included in this experiment. Infestation levels were controlled by introducing different numbers of adults into each cage. The three rates of release of adults were 2, 6 and 15 moths per cage per week over a period of 3 weeks. In the two lower levels, the moths were sexed and equal numbers of males and females were released each time. When 15 moths were released per cage, they were selected at random. This procedure resulted in the release of a total of 6, 18 and 45 moths per cage in treatments 1, 2 and 3, respectively. The releases were made over a 3-week period to insure a uniform level of infestation in each treatment.

The plots were caged June 4 and treated with aldrin to destroy any boll weevils that may have been present. All squares were hand-picked at the time of caging the moths to insure uniform fruiting so that the different levels of infestation would be evident. Moth releases were made June 18, June 24 and July 2. Infestation records were taken at 5- to 7-day intervals from June 30 to August 1, inclusive. Records of infested blooms were obtained by counting total blooms and recording the percentage of rosetted blooms in each plot. Boll infestation records were started July 10 when bolls were 2 to 3 weeks old. A sample of 25 bolls was collected from each plot at weekly intervals, cut open and examined for the number of larvae. The boll infestations were recorded as percentage bolls infested and number of larvae per infested boll.

The plots were defoliated August 6 with Aminotriazole and cotton snapped on August 22. The cotton was ginned in the Cotton Section Laboratory of the Texas Agricultural Experiment Station. A power-operated, 20-saw gin with cleaning equipment for ginning snapped cotton was used. A small sample of lint was sent to the Cotton Ginning Research Laboratory at Mesilla Park, New Mexico for the various fiber tests. The remainder of the lint was shipped to the ACCO Fiber and Spinning Laboratory in Houston, Texas for staple, grade, spinning tests and valuation. Seed samples were tested by the Cottonseed Products Research Laboratory at Texas A. and M. College.

Results.—The rate of pink bollworm infestation buildup is shown in table 1. These records indicated a very uniform progression of increase in rosetted blooms until green bolls were available after which the rate of infestation became erratic. The percentage of infested bolls was relatively high at the time of the first count and, by the second count, the bolls in all treatments were com-

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[3] Texas Agricultural Experiment Station.

Table 1.—Bloom and boll infestations in evaluation studies of pink bollworm damage to cotton at College Station, Texas, 1955.

Number of Moths	Bloom Infestation											
	June 30		July 5		July 10		July 18		July 25		August 1	
	Total Blooms	Per Cent Infested	Total Blooms	Per Cent Infested	Total Blooms	Per Cent Infested	Total Blooms	Per Cent Infested	Total Blooms	Per Cent Infested		
6	124	3.2	201	4.0	156	6.0	53	21.6	6	33.3		
18	113	13.3	191	25.1	163	27.7	76	13.9	15	24.6		
45	106	26.5	181	45.7	156	49.0	73	26.0	18	48.6		
Check	109	1.8	186	0.0	165	0.0	64	4.7	14	0.0		
	Boll Infestation											
					Per Cent Infested	Larvae per Boll	Per Cent Infested	Larvae per Boll	Per Cent Infested	Larvae per Boll	Per Cent Infested	Larvae per Boll
6					32	0.8	86	2.0	96	4.1	98	6.4
18					61	1.4	91	2.5	97	6.2	100	9.6
45					79	2.0	92	3.2	100	9.5	100	12.9
Check					0	0.0	7	0.4	11	1.8	17	1.9

Table 2.—Results of lint tests in evaluation studies of pink bollworm damage to cotton at College Station, Texas, 1955.

Number of Moths	Uniformity Index	Fiber			Staple	Grade
		Strength	Maturity	Fineness		
6	74	98	67	3.6	13/16 to 15/16	O to GO
18	72	99	61	3.2	Perished to 13/16	BG to O
45	72	94	54	3.2	Perished to 13/16	BG to O
Check	77	103	72	4.0	29/32 to 15/16	GOEW to SGOEW
L.S.D. at 5% level	3.1	5.5	5.1	0.3		

pletely infested. The number of larvae per boll was considered the most reliable criterion to indicate the infestation level. In this study when the infestation reached approximately four larvae per boll, 100% of the bolls were infested. Thus, the percentage infested bolls could not be used to determine the insect population in heavy infestations. The infestation reached an average of 6.4, 9.6 and 12.9 larvae per boll in the light, medium and heavy infested plots, respectively.

Results of the lint tests are shown in table 2. The differences between treatments in the fiber tests were significant. The grade and staple were also lowered significantly by the insect damage. Quality of the lint varied from below grade in the heavy and intermediate treatments to strict good ordinary extra white in the check. Staple varied from perished to 15/16 inch in the same order.

The various tests on the seed and results obtained are shown in table 3. The differences in all tests were highly significant. These results indicate that each level of pink bollworm infestation caused a decrease in the quality of seed. Most of these differences between treatments are significant.

Table 4 shows the differences in yields and the value of lint and seed cotton per acre from each treatment. The yield of snapped cotton was not significantly decreased by the insect damage. However, after ginning, the difference in lint yield between the check and heavy infestation was significant and difference in seed yield between the same two treatments was highly significant. These differences, becoming evident after ginning, resulted from the partially-opened, insect-damaged bolls being separated and discarded as trash during the ginning operation. In the check, there was a 30.8% loss in trash compared with 59% in the heavily infested treatment.

The difference in value of the lint and seed produced in each treatment was outstanding. The loss in value of the cotton in each treatment caused by reduced yield and reduced quality is given in figure 1. The total value of seed and lint per acre for each level of infestation was as follows: heavy infestation, $50.36; intermediate infestation, $91.95; light infestation, $131.42; and check, $162.69. The value of lint in the heavily infested plots represents about a 200% reduction in value compared with the check. The differences in the value of seed were even greater. The seed produced in the check was worth over 500% more than the seed from the heavy level of infestation.

These large differences in value were caused by reduction in both yield and quality of the cotton. Line A in figure 1 was obtained by figuring the value of cotton produced at all infestation levels at the same price paid

Table 3.—Results of seed tests in evaluation studies of pink bollworm damage to cotton at College Station, Texas, 1955.

Number of Moths	Per Cent						Quantity Index	Quality Index	Grade
	Dirt and Trash	Free Fatty Acids	Oil	Ammonia	Protein	Lint			
6	3.1	2.8	13.3	4.1	21.0	14.3	76.21	97.55	81.45
18	4.2	4.6	9.7	3.7	19.0	17.2	52.42	91.87	48.38
45	5.6	6.7	7.3	3.3	17.0	19.4	35.53	93.74	33.35
Check	1.8	0.4	15.1	4.7	24.1	11.4	90.65	99.20	90.00
L.S.D. at 5% level	0.7	1.8	2.2	0.4	2.1	2.4			

Table 4.—Yield, gin waste and value of cotton produced at various levels of pink bollworm infestation at College Station, Texas, 1955.

Number of Moths	Pounds per Acre			Per Cent Lint	Per Cent Seed	Per cent Loss in Trash	Value per Acre		
	Snapped Cotton	Lint	Seed				Lint	Seed	Total
6	2350	512	813	21.5	34.5	44.0	116.31	15.21	131.52
18	2200	457	695	20.7	31.7	47.6	85.04	6.91	91.95
45	1650	263	407	16.5	24.5	59.0	47.37	2.99	50.36
Check	2150	551	951	25.5	43.7	30.8	143.61	19.08	162.69
L.S.D. at 5% level		159.6	251.8						

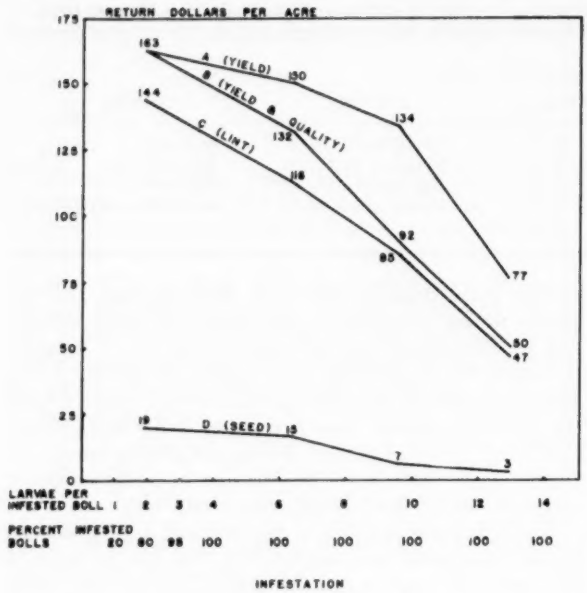

FIG. 1.—Dollar losses per acre from reduced yields and lowered quality caused by pink bollworm damage at College Station, Texas, 1955.

for the check. The difference in returns reflects only losses caused by reduced yields. Line B shows losses caused by both yield and quality reduction. Thus, the differences in line A and line B are a measurement of loss caused by reduced quality. With 100% of the bolls infested and an average of 6.4 larvae per boll, reduction in cotton quality amounted to $18 per acre and yield losses were $13 per acre. At the intermediate level of infestation, 9.6 larvae per boll caused per acre losses of $42 for quality and $29 for yield. Most of the loss at the heavy level of infestation with 12.9 larvae per boll was caused by reduced yield.

SUMMARY.—Heavy pink bollworm infestations cause large reduction in the value of cotton. With 100% of the bolls infested and averages of 6.4 and 9.6 larvae per boll, most of the loss in value of the cotton was caused by reduced quality. When the infestation reached 12.9 larvae per boll, reduction in yield accounted for more of the loss than quality. Two considerations should be remembered in the evaluation of the results of this experiment. First, the pink bollworm infestations used here are extremely heavy. Infestation levels in the field approaching that of the lightest level in this test were rarely found and then only after insecticide treatments had been stopped late in the season. Second, this test was conducted under dry weather conditions which may have minimized pink bollworm damage to cotton. The first pink bollworm emergence holes were found in bolls collected from the plots July 18. Subsequent to this date, less than 1 inch of rainfall was recorded until the cotton was harvested. It is quite possible that yield and quality might have been further reduced by boll rots and water damage to the lint had wet weather conditions prevailed during this period.

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Ovarian Development of Worker Honey Bees[1]

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The female larva of the honey bee can develop into either a worker or a queen, depending upon the food received during larval development. Neither the nutritional factors responsible for dimorphic development nor the mechanisms by which nurse bees control the nutrition are understood.

The literature on the chemistry of the foods of queen and worker larvae has been reviewed by Johansen (1955) and Ribbands (1953). The food of queens is especially rich in pantothenic acid and biotin. Rhein (1951) mixed large amounts of individual vitamins in the food of worker larvae, and reported that the adults reared on the foods had greatly enlarged ovaries when pantothenic acid was added to the diet, slightly larger ovaries when biotin was added to the diet, and that other vitamins produced less marked effects. No details of the results were given. Kuwabara (1947) observed that worker larvae were fed more often, and many of them developed into adults with more ovarioles, in a queenless colony than in a queenright colony. Levin & Haydak (1951) studied seasonal fluctuations in the number of ovarioles in the ovaries of worker bees. They were unable to correlate any of the probably non-significant fluctuations with environmental influences. Haydak (1943) showed that undernourished queen larvae will develop into intermediates between queens and workers.

MATERIALS AND METHODS.—Commercial strains of Italian honey bees (*Apis mellifera* L.) were used in these studies. Two small colonies of bees were placed in cages and deprived of pollen. When the bees were no longer able to rear larvae, one colony was given a pollen cake made of approximately 60% trapped, air-dried, pollen and 40% water; the other colony was given a similar cake to which 250 μg./gm. of Ca·pantothenate had been added. This experiment was repeated three times. Further experiments were conducted in early March on uncaged colonies of about the same strength that had not reached their peak populations. Each queen was re-

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stricted to one empty comb for a day, then this comb of eggs was moved above a queen excluder with about half of the brood. The queen was kept below the excluder with the other half of the brood, or she was removed from the colony. When the queens were removed from the experimental combs of eggs, a colony was given one of the following treatments: (1) a colony was kept normal to serve as a control; (2) all eggs and larvae, except those in the experimental comb, were removed from a colony, and each 1 to 3 days thereafter all newly laid eggs were removed before they hatched; (3) a colony was made queenless and given the eggs from (2); (4) a colony was made queenless and was not disturbed again; (5) a colony with a double grid pollen trap and with little pollen was given a pollen cake; (6) a similar colony was given a pollen cake containing 600 μg./gm. of Ca·pantothenate. This entire experiment was repeated with an additional treatment: (7) a colony with a pollen trap and little pollen was given a pollen cake containing 10 μg./gm. of biotin.

The treatments were discontinued when the larvae in the experimental combs had been sealed. Three days before the adults were due to emerge the combs were moved to cages in a constant temperature cabinet held at 93° ± 1° F. Immediately after they emerged, adults were quick frozen and held in a deep freeze until ready for dissection. In two of the experiments some of the adults were marked with a spot of paint and returned to the colony for 3 weeks before being dissected, and some of the adults from one colony were held in the constant temperature cabinet for 3 weeks.

The last abdominal segment of a bee was grasped with forceps, torn loose, and pulled steadily until the attached intestine was removed from the abdomen. A mid-dorsal incision was then made in the abdomen, and the ovaries were floated on a droplet of water. After they had been teased apart with needles, the ovarioles usually could be counted at 41× magnifications while still in the abdomen, but if the ovaries were large they were removed to a glass slide, the distal end was cut off, and the ovarioles were separated and counted. The genae and frons were cut from the remainder of the head capsule, and the length of one mandibular gland was measured at 16× magnifications from its distal end to the base of the mandible. The tongue was measured at the same magnification from the distal end of the paraglossae to the tip.

The difference in measurements of bees was determined by the "t" test or by an analysis of variance. These methods, and the method of fitting a Poisson frequency distribution, were taken from Snedecor (1946).

RESULTS AND DISCUSSION.—In the first experiment with colonies of bees in cages, no adults emerged from the colony which received a normal pollen cake; the silk of their cocoons and the last larval skin clung to both the bees and the cells and prevented emergence. This seems to be a nutritional deficiency symptom which has also been observed during attempts to rear larvae in caged colonies on a chemically defined diet. In the second experiment the bees could not be dissected because an imperfect method of preserving the adults was used. In the third test there was no significant difference in the number of ovarioles in the ovaries of bees that had received added Ca·pantothenate during larval life and those that had not.

In the first experiment with colonies in the field (Experiment 4), most of the bees old enough to fly drifted from the two queenless colonies into the check colony which stood between them, and on the third day of development the experimental larvae in the queenless colonies were inadequately fed. The combs containing these larvae were moved to a higher concentration of bees below the excluders where they were given sufficient food during the remainder of their development. Since these larvae were underfed for about a day and the colony that was intended as a check had a low larval/nurse bee ratio, only a few bees from each treatment were preserved and dissected. Adults reared during the treatments differed significantly between colonies in tongue length, mandibular gland length and number of ovarioles in the ovaries. In each of these characteristics the bees from the check colony were the most queen-like. In tongue length and ovariole number the bees from the two queenless colonies were the most worker-like. Data on the mean number of ovarioles per bee are given in table 1. It is not certain that any of the anatomical differences among the bees reared during these treatments were caused by environmental influences. Worker bees reared in normal colonies from eggs laid 7 months after the experiment by two of these queens both had means of 12.8 ovarioles per bee, based on 39 and 29 bees. These means did not differ significantly from the means of 14.4 and 10.0 ovarioles per bee reared from the same queens during the treatments. Since the other queens had been replaced as soon as the treatments were completed, their normal offspring could not be studied. Normal worker bees from 11 queens other than those used in this experiment have been dissected, however, and all have had means of between 5 and 9 ovarioles per bee.

A similar test (Experiment 5) was conducted in another apiary and with another commercial strain of bees in March of the following year. In this experiment the colonies were widely spaced among trees and shrubs to minimize drifting. There were no visible differences among colonies in the amount of food in the cells of the experimental larvae.

Just before the colony treatments were begun, about 30 teneral bees were removed from each colony to serve as internal controls. The ovarioles were counted in the ovaries of the internal control and of over 100 bees reared during the treatments. The mean number of ovarioles per bee reared before and during the treatments are given in table 1, and figure 1 shows the frequency distribution of ovariole numbers. There were no significant differences between colonies or between bees reared before and during treatments. The bees from the high biotin diet had the highest mean number of ovarioles and differed the most from their internal controls. As can be seen from figure 1, none of the bees reared during this treatment had more than 18 ovarioles, but an unusually large percentage of them had 9 to 14 ovarioles.

From these preliminary experiments it is obvious that the bees used in these experiments could control the direction of differentiation of larvae rather closely. It has been shown that workers will develop on a food that

Table 1.—Mean number of ovarioles in both ovaries of worker honey bees reared throughout larval life in colonies treated as shown, and in the bees reared before the treatments in one experiment.

Treatment	Experiment 4		Experiment 5			
	Reared During Treatments		Reared During Treatments		Reared Before Treatments	
	No. Bees	No. Ovarioles	No. Bees	No. Ovarioles	No. Bees	No. Ovarioles
Normal controls	10	14.4	109	6.5	29	6.1
Low larval/nurse bee ratio	24	10.0	114	7.5	27	7.3
Queenless, but eggs and larvae	19	6.7	116	7.2	32	6.6
Normal queenless	23	6.6	113	7.6	35	6.8
Normal pollen cake	20	11.5	114	7.2	33	6.6
High Ca-pantothenate	15	10.9	115	7.1	40	6.5
High biotin	—	—	134	8.2	35	6.7

will not produce queens (Weaver 1955), but it has not been definitely proved that there is a biologically significant difference in the bee milk (Ribbands 1949) fed to the larvae of queens and workers. Unpublished results from this laboratory, however, strongly indicate that such a difference exists, and that for the differentiation of queens, both large amounts of food and food of a special quality are required. If this is the case, workers would be produced from foods that varied rather widely either in quality or quantity, providing the other were kept under control. The bee with 70 ovarioles from the high pantothenic acid diet, and the bee with 130 ovarioles from the queenless colony that was given eggs, could have been fed as queens during a part of their larval life and as workers during the remainder of their development. It is also possible that they received one or more feedings con-

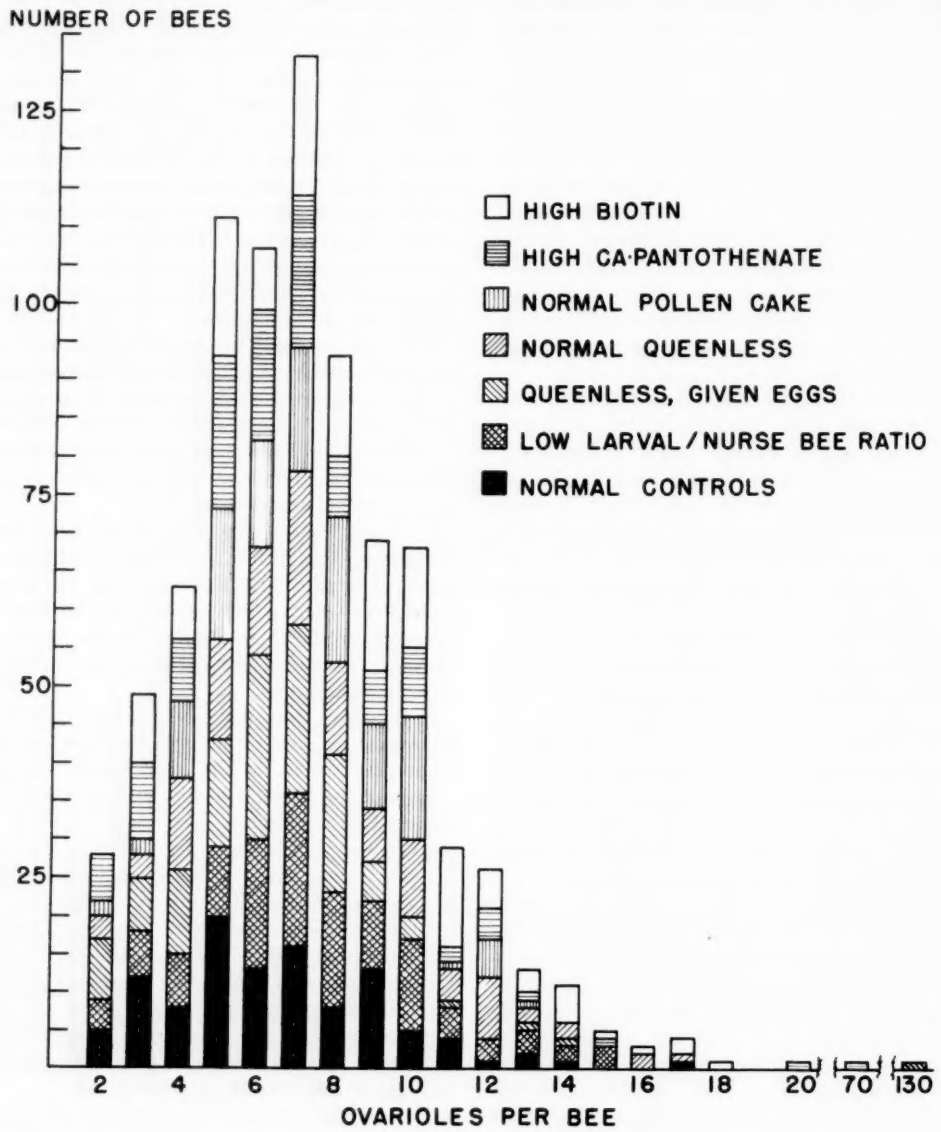

FIG. 1.—Frequency distribution of number of ovarioles in both ovaries of worker honeybees reared during larval life in colonies receiving treatments as indicated.

taining large amounts of the substance(s) necessary for the development of queens, that they were more sensitive than other larvae to the same amount of the substance(s), or that they received more food than the other larvae and thus were able to develop larger ovaries on approximately the same amount of the substance(s). These possibilities will have to be studied by other techniques.

In view of the results obtained by Kuwabara (1947) it seems possible that different races or strains of bees might differ in their ability to control the direction of differentiation of their larvae. Because of the negative findings in this study and the uncertainty of the general applicability of the results, no definitive experiment was undertaken.

Even the bees in weak colonies in cages always started queen cells when fed a pollen cake containing large amounts of Ca·pantothenate. The queen cells were torn down after they were sealed and the treatment was discontinued. This suggests that pantothenic acid may be a limiting factor in the production of royal jelly, the bee milk fed to queen larvae. The role of the large amount of pantothenic acid in royal jelly is not known; it could be of importance in the differentiation of queens, a side effect of a high requirement for pantothenic acid by the brood food glands, or occurrence of no biological significance. The last alternative seems unlikely, but at present there are no good techniques for testing these possibilities.

As will be noted from figure 1, the data on ovarioles per bee follow the approximate form of a Poisson frequency distribution; the number of ovarioles in the individual ovaries follows the Poisson distribution slightly more closely. There was a mean of 0.15 more ovarioles in the right ovary than in the left ovary of these bees. When the number of ovarioles in the left ovary of each bee is compared with the number in the right ovary of the same bee, a plot of the data has the approximate form of a normal curve. In 19% of the bees the right and left ovary had an equal number of ovarioles, 37% differed by one ovariole, 21% by two, 13% by three, 7% by four, and 3% by five or more ovarioles. Approximately an equal number of the ovaries had odd and even numbers of ovarioles. Teneral bees did not differ significantly in ovariole numbers from 3-week-old bees produced in the same colony at the same time.

SUMMARY.—Colonies of honey bees were subjected to several treatments in an attempt to cause the nurse bees to alter the quality or quantity of the food given to the larvae, and the ovarian development of the adults reared during the treatments was studied. Most of the bees reared during the treatments were completely worker-like, and the only significant differences between colonies in the mean number of ovarioles per bee may have been caused by genetic rather than environmental factors. Only two worker bees reared during these treatments had an unusually large number of ovarioles; the possible causes of the excessive ovarian development by these bees are discussed. Since colonies given a pollen cake containing 250 to 600 μg./gm Ca·pantothenate always started queen cells, it is proposed that pantothenic acid may be a limiting factor in the production of royal jelly by nurse bees.

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Transmission of Resistance Between Strains of Two-Spotted Spider Mites[1]

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That mating may occur between closely related species of tetranychid mites was reported by McGregor & Newcomer (1928) in their studies with *Paratetranychus citri* (McG.) and *Metatetranychus ulmi* (Koch). However, fertilization apparently did not take place, as the mated females produced only males, which in this group normally come from unfertilized eggs. More recently Newcomer (1954) concluded, on the basis of similar cross-mating experiments, that *Tetranychus pacificus* McG. and *T. mcdanieli* McG. are also distinct species.

Keh (1952) obtained progenies of both sexes in crossing males of *Tetranychus multisetis* McGregor and females of *T. telarius* L. (=*bimaculatus* Harvey) and also in the reverse crossing, which indicated that the females were fertilized. Most females of the F_1 generation were sterile, but a few continued to be fertile beyond the F_2 generation. On the basis of his biological and morphological studies, Keh concluded that *multisetis* is a subspecies of *telarius*.

Preliminary experiments in cross-mating individuals from three strains of two-spotted spider mites originating from greenhouse crops and identified at various times as *Tetranychus bimaculatus* Harvey[3] are described herein.

One of these strains, referred to as the Beltsville colony, is characterized by the carmine color of the females, with four dark body spots laterally, the anterior pair being larger than the posterior pair near the anal area. Coloration is assumed a few days after emergence. This colony has been maintained since 1938 from a natural infestation on greenhouse cucumber at Beltsville, Md. The second strain, called the Massachusetts colony, was collected from carnation near Waltham in October 1951. The third strain, the Cranbury colony, was obtained from roses in New Jersey in February 1949. Adult females in the last two colonies are green, occasionally yellowish or orangish with brown spots, with two dark spots at each side of the middle of the body. The Beltsville and Massachusetts colonies are nonresistant to organic phosphorus compounds whereas the Cranbury colony is resistant. All stock colonies have been maintained on lima beans without exposure to acaricides. The results of these experiments provide information on the inheritance of resistance to acaricides in the Cranbury colony and the red coloration found in females of the Beltsville colony.

EXPERIMENTAL PROCEDURE.—Individual quiescent females were isolated with males on lima bean plants that had been trimmed to one leaf with the petiole banded with lanolin. These plants were held in 4-ounce bottles containing a nutrient solution and could be maintained for several weeks unless damaged by mite feeding. A 1-inch barrier of the sticky-band product Deadline around the neck of each bottle prevented contamination from migrating spider mites. Approximately 10 days after the females emerged as adults, they were transferred together with the surviving males to another bean plant, to prevent them from mating with their offspring, the emerging males of the F_1 generation.

In haploid arrhenotokous parthenogenetic species the fertilized eggs of a female develop into diploid females and the unfertilized eggs into haploid males. Therefore back-crosses between the Massachusetts and Cranbury strains were made by allowing the offspring of a given female (haploid males and F_1 females) to mate at random. The offspring of these back-crosses consisted of backcross females and a mixture of haploid males, which carry the genetic characters of either grandparent.

The test for resistance was established from a series of dip tests with various dilutions of malathion ranging from 1:100 to 1:40,000 against the parent stock of resistant and nonresistant mites. The LD 50's for the nonresistant strains were determined to be between 1:10,000 and 1:20,000 and for the resistant strains between 1:100 and 1:200. A 1:5,000 dilution consistently gave 100% mortality of the nonresistant strains and no mortality of the resistant strain. Therefore, this dilution was used to test the resistance of the various progenies.

RESULTS—*Transmission of resistance.* Table 1 indicates that the F_1 females from all crosses and the haploid male progeny from the cross between a resistant female and a nonresistant male are resistant, whereas all the haploid males of the nonresistant female and resistant male crosses were killed. These data indicate that the haploid males have the same degree of resistance as the maternal parent, and, as expected, are similar to the progenies of the virgin females (all males). Since all the F_1 females resulting from crosses involving the resistant strain were resistant, characters for resistance in the Cranbury colony are transmitted by either sex, and there is no indication of maternal effects.

Apparently the F_1 female progeny of the crosses between males or females of the Beltsville colony and matings of the opposite sex of the Massachusetts and Cranbury colonies were sterile, since no eggs have been produced. These results indicate that the sterile F_1 females of the crosses with the Beltsville colony are hybrids and that two species of spider mites are involved.

In contrast to the results obtained from the Beltsville-Massachusetts and Beltsville-Cranbury crosses, the F_1 females of the Massachusetts-Cranbury crosses produced eggs in abundance. They developed into both males and females, which have been used in the production of an F_2 generation or in back-crosses with individuals of their respective parents. These results indicate that the Massachusetts and Cranbury colonies represent strains of the same species.

In crosses between F_1 females of the Cranbury-Massa-

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[2] The writers appreciate the advice and suggestions of S. L. Emsweller, Horticultural Crops Research Branch, U.S.D.A., and of James Crow, Department of Genetics, University of Wisconsin, during this investigation and the preparation of the manuscript.

[3] E. W. Baker, A. Earl Pritchard, and the late H. E. Ewing made critical studies of numerous samples of these mites submitted to them at different times.

Table 1.—Effect of malathion 1:5,000 dip on the progeny of spider mites.

Strain		Number of Offspring Tested	Per Cent Mortality in 24 Hours
Stock colonies:			
Nonresistant (*T. cinnabarinus*)	Females	347	98
Resistant (*T. telarius*)	Females	885	1
Isolated virgin females:			
NR	Males	30	100
R	Males	39	0
T. cinnabarinus NR×*T. telarius R*			
NR female×R male	Males	26	100
	Females	33	0
R female×NR male	Males	44	4
	Females	98	3
T. telarius NR×*T. telarius R*			
NR female×R male	Males	12	100
	Females	92	1
R female×NR male	Males	8	12
	Females	34	0
T. cinnabarinus NR×*T. telarius NR*			
cinnabarinus female×NR male	Males	7	100
	Females	21	100
NR female×*cinnabarinus* male	Males	25	100
	Females	40	100

Table 2.—Effect of malathion 1:5,000 dip on the back-cross progeny of resistant F_1 females.

Back-Cross		Number of Offspring Tested	Per Cent Mortality in 24 Hours
Female	Male		
NR female	RF_1×NR	Females 240	47.9
R male		Males 59	49.2
R female	RF_1×R	Females 123	2.4
NR male		Males 12	18.7

chusetts matings and haploid males from a resistant maternal parent, the backcross females were resistant (table 2). F_1 females of the Massachusetts-Cranbury crossed with haploid males from a nonresistant maternal parent produced backcross females that were resistant and nonresistant in a 1 to 1 ratio. This indicates that resistance is a dominant characteristic and probably involves a simple factor.

The progeny from the crosses of the Cranbury and Massachusetts colonies represent strains of the same species. At the suggestion of E. W. Baker, examples of the three colonies of mites and progeny resulting from the various crosses were submitted to H. Bruce Boudreaux, of Louisiana State Univeristy, who has discovered morphological characters for differentiating *Tetranychus telarius* from closely related species (Boudreaux 1956). Dr. Boudreaux identified the mites of our Beltsville colony as *T. cinnabarinus* (Boisd.) and the F_1 progeny as typical hybrids with *telarius* represented by our Massachusetts and Cranbury colonies. The present biological tests confirm his findings, published after the completion of our studies reported herein.

Transmission of red coloration.—Keh (1952) cross-mated the carmine females of *Tetranychus multisetis* and green males of *bimaculatus* as well as reverse crosses, obtaining carmine females in the F_1 generation of both series. This indicated a genetic dominance of the carmine color over green and the transmission of this character by either males or females.

In our studies the sterility of the F_1 females resulting from crosses with *cinnabarinus* females of the Beltsville colony and *telarius* (=*bimaculatus*) males of the Massachusetts or Cranbury colonies or the reverse crosses indicates a more distant taxonomic relationship. However, the relationship is close enough so that fertilization of the eggs occurred and the factors for carmine color in females and for resistance to acaricides were transmitted to the F_1 female progeny. These F_1 females usually do not have the dark spots of the parents. Since the male offspring were from unfertilized eggs, they were the same as the female parent.

The results of this preliminary work with two strains of *telarius* indicates that resistance is a dominant characteristic and a simple factor may be involved. The character for resistance is transmitted by either sex.

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The Effect of Certain Acaracides on Two Species of Spider Mites on Cotton[1]

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Spider mites are in general most numerous during seasons of hot, dry weather (Arant 1951). The past several years have been extremely dry in Alabama and consequently mite infestations have been above average. The destruction of natural enemies by the widespread use of organic insecticides may have been another contributing factor to mite abundance. The principal species noted were the desert spider mite, *Tetranychus desertorum* Banks,[2] and the strawberry spider mite, *T. atlanticus* McG.[2]. The two-spotted spider mite, *T. telarius* (L.),[2] was also present on cotton in some localities in northern Alabama but caused little, if any, economic damage.

Spider mites on cotton have been studied by many investigators. Roussell *et al.* (1951) reported that infestations of the tumid spider mite, *T. tumidus* Banks, at Baton Rouge, Louisiana, caused a 45% reduction in the yield of seed cotton. Studies on the effectiveness of acaracides on different species of mites under various conditions have been made by Melvin & Earle (1948), Newcomer & Dean (1949), Neiswander *et al.* (1950), Smith & Bryan (1951), Mistric & Rainwater (1952), and others. Effectiveness of acaracides on cotton was found to vary with the species of mite and the levels of infestation.

Field experiments for chemical control of spider mites on cotton were conducted at three localities in Alabama during 1954. The results of these experiments are herein reported.

Experiments with Desert Spider Mite.—Two experiments were conducted to determine the effects of several acaracides on the desert spider mite. One was conducted at Headland in southeastern Alabama, using dust applications. The other experiment was at Deatsville in central Alabama, where the acaracides were applied as sprays.

Procedure.—In the experiment with dusts, plots consisted of 8 rows of cotton 70 feet long with 2 untreated buffer rows between plots. Tiers of plots were separated by 10-foot alleys. Treatments consisted of seven acaracidal dusts and a check. All treatments were replicated four times in randomized blocks. The dusts were applied with a rotary-type, 1-row hand duster adjusted to deliver approximately 20 pounds per acre per application. Applications were made when the air was relatively calm. Infestation counts of mites were made prior to treatment and 1, 2, 4, 5, 7, and 15 days after the initial treatment of July 22.

In the experiment at Deatsville with sprays, treatments consisted of six acaracides and a check. All treatments were replicated four times in randomized blocks of plots. Plots were 6 rows wide and 69 feet long. Sprays were applied with a 6-row high-clearance, self-propelled sprayer calibrated to deliver 6 gallons per acre per application. Infestation counts of mites were made prior to spraying and 24 and 48 hours after treatment.

In both experiments, infestation counts of mites were made on 20 leaves collected at random from the inner four rows of each plot. The fourth leaf from the terminal bud was used in all counts. The leaves were removed from the plants, placed in a paper bag, and taken to the laboratory for determination of infestation. The number of mites on 1 square inch of leaf surface near the mid rib of each leaf was counted with the aid of a binocular microscope. Counts of adult and immature mites were combined.

Results.—Results of the experiments with acaracidal dusts are presented in table 1. All materials tested were effective in reducing the population of the desert spider mite to a level significantly lower than that on the check plots. All organic acaracides were significantly more effective than sulfur 48 hours after application of the dust. The infestation increased the third and fourth days following treatment to such an extent that a second application was necessary on July 26. Heavy rainfall immediately following the dusting made it necessary to repeat the application of July 28. All organic acaracides were again more effective in reducing the mite infestation than was sulfur. No material gave a complete knockout of mites, and no material exhibited any appreciable ovicidal action. Five per cent Aramite had the longest residual effectiveness of any material tried. About 1 week following the second dusting, the population of mites declined on all plots. The cotton was maturing at that time, and many of the leaves were falling from the plants.

Table 1.—Numbers of desert spider mites on cotton before and after treatment with acaracidal dusts, four replications, Headland, 1954.

Treatments[a]	Average Number of Mites per Square Inch					
	7/21	7/23	7/24	7/26	7/29	8/5
Check	30.1	21.3	31.9	37.2	6.1	1.9
Aramite, 3%	35.9	2.8	11.6	13.4	0.2	0.2
Aramite, 5%	41.9	1.3	3.6	6.8	0.2	0.2
Malathion, 5%	38.3	2.2	11.7	18.8	0.8	0.8
Chlorthion, 3%	28.4	2.0	10.7	20.8	0.1	1.3
Methyl parathion, 3%	23.3	1.6	5.5	14.8	1.5	0.3
Sulphur, 97%	31.7	7.5	13.4	24.1	4.7	3.0
Parathion, 1%	30.1	1.0	3.3	10.1	0.2	1.0
L.S.D. at 5% level	—	3.30	11.94	10.09	0.77	n.s.
L.S.D. at 1% level	—	4.48	16.25	13.77	1.04	n.s.

[a] Dusted July 22, July 26 (followed immediately by 3 inches of rain), July 28.

Results of the experiment with sprays are presented in table 2. All sprays were effective in reducing the mite infestation below that of the check plots. There was no significant difference among effectivenesses of acaracides. There was considerable reduction in the population of mites on the check plots and in other untreated areas in the field. A predaceous thrips, *Scolothrips sexmaculatus*[2] (Perg.), was observed feeding on the mites in these areas; however, it is doubtful if this predator was important in causing the decline in population. As a result of the de-

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Table 2.—Numbers of desert spider mites on cotton before and after treatment with acaracidal sprays, four replications, Deatsville, 1954.

Treatments[a]	Pounds Technical per Acre	Average Number of Mites per Sq. Inch		
		8/4	8/5	8/6
Check	—	15.9	4.7	4.4
Ovex	2.00	24.9	0.5	1.1
Demeton	0.25	13.5	0.1	0.2
Methyl parathion	.25	17.3	0.4	1.3
Parathion	.25	10.0	0.2	0.8
Chlorthion	.50	24.0	0.5	0.2
Malathion	.50	12.3	0.6	1.2
L.S.D. at 5% level		—	2.77	2.52
L.S.D. at 1% level		—	3.79	3.45

[a] Sprays applied after counts completed on August 4.

clining population, it was not feasible to continue the experiment beyond the third day.

EXPERIMENT WITH STRAWBERRY SPIDER MITE.—A field experiment to determine effectiveness of acaracidal sprays on the strawberry spider mite was conducted in northern Alabama. The treatments and plot layout were the same as those used at Deatsville. Infestation counts of mites were made prior to spraying and at intervals after treatment. Infestation counts were made on 20 leaves per plot as previously described.

Treatments, rates of material per acre, and spider mite infestations are summarized in table 3. All materials gave excellent control of the strawberry spider mite 24 and 48 hours and 6 days after spraying. There were no significant differences among acaracides. All material continued to give good control of mites 6 days after treatment. A decline in the mite population after August 30 prevented determination of further residual action of the materials. This decline of mites was associated to some extent with the maturation of the cotton.

SUMMARY.—The desert spider mite, *Tetranychus desertorum* Banks, and the strawberry spider mite, *T. atlanticus* McG., were abundant on cotton in several localities in Alabama in 1954. The two-spotted spider mite, *T. telarius* (L.), was of minor importance.

In replicated small-plot experiments, 3 and 5% Aramite, 3% Chlorthion, 5% malathion, 3% methyl parathion, and 1% parathion applied as dusts at the rate of 20 pounds per acre were more effective than sulfur in controlling the desert spider mite. None of the materials gave complete control with a single application, but 5% Aramite had a longer residual effect than the other materials. Sprays were effective in reducing infestations of desert and strawberry spider mites when applied at the following rates per acre: Chlorthion, 0.5 pound; demeton, 0.25 pound; malathion, 0.5 pound; methyl parathion, 0.25 pound; parathion, 0.25 pound; ovex, 2.0 pounds. Hercules AC 538 at 0.25 pound of technical material per acre gave good control of strawberry spider mite in northern Alabama.

Table 3.—Numbers of strawberry spider mites on cotton before and after treatment with acaracidal sprays, four replications, Crossville, 1954.

Treatments[a]	Pounds Technical per Acre	Average Number of Mites per Sq. Inch			
		8/23	8/25	8/26	8/30
Check	—	10.9	11.6	14.0	15.6
Ovex	2.00	10.2	1.4	0.8	0.2
Demeton	0.25	6.1	0.1	0.5	0.0
Methyl parathion	0.25	4.5	0.4	2.6	0.8
Parathion	0.25	5.7	0.1	0.3	1.6
Chlorthion	0.50	9.8	1.0	3.1	1.7
Malathion	0.50	12.1	0.3	1.0	3.9
Hercules AC-528[b]	0.25	10.7	1.7	1.8	1.7
L.S.D. at 5% level	—	—	4.25	3.31	6.39
L.S.D. at 1% level	—	—	5.79	4.50	8.72

[a] Sprays applied on August 24.
[b] 2,3-*p*-Dioxanedithiol S,S-bis (*O,O,*-diethyl phosphorodithioate).

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Temperature and the Action of Pyrethrum in the American Cockroach[1,2,3]

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Increased toxicity of pyrethrum at low temperatures has been reported for several insect species. *Circulifer* (*Eutettix*) *tenellus* (Baker) (Harries *et al.* 1945), *Musca domestica* L. (Chamberlain 1950), *Blattella germanica* (L.) (Guthrie 1950), *Macrodactylus subspinosus* (F.). (Hartzell & Wilcoxon 1932) and *Apis mellifera* L. (Bottcher 1938–9) show increased susceptibility to pyrethrum at lower temperatures. Although a negative temperature coefficient of action is known for several insecticides, pharmacological and biochemical considerations of this phenomenon have received very little consideration.

Vinson & Kearns (1952) investigated the negative temperature coefficient of DDT in the American cockroach (*Periplaneta americana* (L.)) and demonstrated that metabolism of DDT proceeded at the same rate at 15° C. as at 35° C. when equivalent dosages were compared. Although DDT penetrated more rapidly at 35° C. the cockroaches were unaffected; those maintained at 15° C. were prostrate. These observations indicate that factors additional to detoxication are present to maintain a normal state in the cockroaches held at the higher temperature.

The complex biochemical picture of the effect of low temperature on poisoning has been examined further by Sternburg & Kearns (1952) who showed that the negative temperature coefficient of toxicity found in the American cockroach was associated with the presence of a toxin other than DDT in the blood of DDT-poisoned cockroaches. The concentration of this toxin could be correlated with the syndrome of the poisoned insect.

After pyrethrum was found to have a negative temperature coefficient in *Periplaneta americana*, possible correlation between toxicity and penetration was examined. Then, relationship between toxic materials in haemolymph and the symptomatic reactions was investigated.

METHODS AND MATERIALS.—A sample of C^{14}-pyrethrum which was biosynthesized by Pellegrini *et al.* (1952) was employed for tracer studies.[5] The material had a specific activity of 132 cpm/μg. The insecticide, which was received as a hexane solution, was redissolved in acetone because of the great toxicity of hexane to insects.

The non-radioactive pyrethrum was purified by chromatographing a technical preparation of 20% pyrethrum in kerosene on carbon. The purified material was made up to a known concentration in glass-distilled acetone and was stored at 3° C. until used.

A middle cut from a fractionation of technical piperonyl butoxide[5] (Miller *et al.* 1952) was employed in synergist studies. Like the pyrethrum, the synergist was prepared as an acetone solution of known concentration.

American cockroaches employed in these studies were reared on dog biscuits (Purina). Adult flesh flies (*Sarcophaga crassipalpus* Macq.) were employed for bio-assay studies. Sarcophagid larvae were reared on pork liver; adult flesh flies received sucrose as a carbohydrate source and liver as a protein source. Because of their long life cycle, flies up to 2 weeks old were used for bio-assay studies. Flies of both sexes were used.

Cockroaches were topically treated by applying a 1-mm.[3] solution of pyrethrum in acetone to the sternum of the cockroach between the prothoracic and mesothoracic coxae. The solution was delivered by means of a syringe-micrometer assembly. A 0.25-ml. calibrated syringe was equipped with a 27-gauge needle, whose tip was bent downward in order to facilitate the even deposition of the droplet on the insect.

Blood was collected from pyrethrum-treated cockroaches in the following manners. After the antennae were cut, slight pressure was applied to the head of the cockroach, causing the blood to exude from the antennal stumps. It was allowed to drop onto a watchglass mounted on a piece of dry ice, and was frozen immediately. Blood was collected also from the cockroach legs which had been severed at their juncture with the body. By cutting through the severed legs in the tarsal region and squeezing the femur, small volumes of blood were obtained. After the blood had been collected, it was placed in a small tube, thawed, and centrifuged (refrigerated) in order to separate the haemocytes. The serum was decanted and refrozen until used.

The presence of toxic substances in the blood of pyrethrum-poisoned cockroaches was detected by injecting the blood into sarcophagid flies and determining the duration of paralysis of the flies. Preliminary tests showed that the flies could tolerate doses of 5 to 10 μl. of normal cockroach blood without exhibiting abnormal symptoms. Since paralysis in many instances was of short duration, the use of an anaesthetic, such as carbon dioxide, was considered disadvantageous. Consequently, the flies selected for treatment were collected individually in 2-inch pieces of glass tubing from the sides of the cage. The enclosed fly was removed from the tube with forceps and injected in the prothorax. Small volumes of blood were delivered from a calibrated syringe which had been drawn from a capillary tube. The posterior portion of the syringe contained mercury which occupied the greater volume of the syringe; therefore, the syringe was capable of accommodating very small volumes of blood. By means of a short length of rubber tubing, the microsyringe was attached to a calibrated 0.25-ml. tuberculin syringe, the plunger of which was driven by a micrometer.

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[4] Quartermaster Research and Development Command, Natick, Massachusetts.

[5] Obtained through the courtesy of Mr. A. C. Miller of the Gulf Research and Development Company, Pittsburgh, Pa.

The rate of penetration of pyrethrum at the two temperatures was examined by employing C^{14}-pyrethrum. Twenty female cockroaches were treated, each with 4 μg. of C^{14}-pyrethrum, and immediately were divided into two equal groups; one group was placed at 35° C. and the other at 15° C. At 2 and 12 hours after treatment, five cockroaches were removed from each group and rinsed three times with 5-ml. portions of diethyl ether and the rinses were combined. The rinses were concentrated on the steam bath, were transferred to 5-ml. volumetric flasks, and were diluted to volume. Duplicate 0.5-ml. aliquots of each rinse were transferred to counting planchets and were assayed for radioactivity 12 hours later on a thin window counter. Zero time controls established the near quantitative recovery of the pyrethrum by this method. Employing C^{14}-pyrethrum, an assay for the possible presence of the insecticide in the blood from pyrethrum-poisoned cockroaches was undertaken. The blood samples were each extracted three times with 1-ml. portions of diethyl ether and twice with 1-ml. portions of ethyl alcohol. The ether and alcohol rinses were combined and the volume was reduced to 1 ml. This method had been shown to quantitatively remove C^{14}-pyrethrum from normal cockroach blood to which the labelled insecticide had been added. Aliquots, 0.5 ml in volume, were transferred to counting planchets which were assayed for radioactivity 12 hours later.

RESULTS AND DISCUSSION.—The 24-hour LD-50 for topical doses was indicated to be about 1 μg. per cockroach at 15° C. or about 6 μg. per cockroach at 35° C. (table 1). These data clearly established the negative temperature coefficient of pyrethrum in the American cockroach.

The results of penetration studies with C^{14}-pyrethrum demonstrated that comparable dosages of pyrethrum penetrated more than twice as fast at the elevated temperature (table 2). The initial rates of penetration were most rapid at both temperatures studied. Recovery of the absorbed pyrethrum was low at both temperatures; 38% of the absorbed material was unaccounted for at 35° C. and 24% at 15° C.

The ability of the cockroach to tolerate relatively high concentrations of pyrethrum was critically associated with its maintenance at a fairly high temperature. If on the other hand, pyrethrum-treated cockroaches were transferred from 35° C. to 15° C., paralysis rapidly ensued. Return of the cockroaches to 35° C. rapidly restored them to a state of apparent normalcy. This process could be repeated for several hours, the authors having alternated the treated cockroaches between the two temperatures for as long as 8 hours, 1 hour being the maximum time a cockroach was held at either temperature. The length of time a cockroach can be held at 15° C. before being transferred to 35° C. in order to dissipate the symptoms of poisoning was contingent on the concentration of applied pyrethrum (table 3). The ability of the cockroach to withstand pyrethrum at low temperatures was extremely limited, mortality being very high if exposure time at 15° C. exceeded 4 hours.

Table 1.—The effect of temperature on the mortality of female American cockroaches 24 hours after topical treatment with pyrethrum.

Number of Cockroaches	Dosage (μg. Pyrethrum)	Per Cent Mortality	
		15° C.	35° C.
20	0.5	10	0
20	1	40	0
20	2	100	0
20	4	—	10
20	6	—	60
20	8	—	80

Table 2.—The effect of temperature on the rate of penetration of C^{14}-pyrethrum in the American cockroach 2 and 12 hours after application of 4 μg. per insect.

Number of Cockroaches	Temperature (° C.)	Time After Application (hr.)	Amount Absorbed (μg.)	Per Cent Penetrated
10	35	2	1.7	43
10	15	2	0.76	19
10	35	12	2.9	72
10	15	12	1.3	32

Table 3.—The effect of temperature on the ability of the pyrethrum-poisoned cockroach at 15° C. to revert to normalcy at 35° C.

Dosage (μg. Pyrethrum)	Condition at 15° C.	Average Time Prostrate at 35° C. After Transfer (Min.)
1	Prostrate	3
2	Prostrate	5
4	Prostrate	9

Results of studies on the rate of penetration of pyrethrum at 15° C. and 35° C. are presented in table 4. Cockroaches treated with 1 μg. of pyrethrum and maintained at 35° C. exhibited no symptoms of poisoning; treated cockroaches which were maintained at 15° C. began to exhibit symptoms of poisoning within 15 minutes and were largely immobilized in 30 minutes. If the cockroaches which had been maintained at 35° C. were transferred to 15° C. nearly complete prostration occurred within 5 minutes. The reversion to normalcy of cockroaches transferred from 15° C. to 35° C. was almost as rapid. Symptoms of poisoning were reversed within 3

Table 4.—The effect of temperature on the rate of penetration of a 1-μg. dosage of pyrethrum topically applied to cockroaches as determined by poisoning symptoms.

Number of Cockroaches	After-Treatment Temperatures	Number Prostrate at Specified Intervals After Treatment (Min.)		
		5	15	30
10	15° C. continuously	0	2	8
10	35° C. for ½ hr., then placed at 15° C.	9[a]	10	10

[a] 5 minutes after transfer from 35° C. to 15° C.

to 9 minutes after transfer to the elevated temperature. The time taken to revert to normalcy is correlated with the concentration of pyrethrum which the cockroach received (table 3). An examination of these data indicated that at 35° C. the toxicant had attained those sites critical to prostration much more rapidly than at 15° C., but at the elevated temperature the insect has the ability to compensate for the presence of the material at these critical centers. This compensatory reaction is extremely efficient as evidenced by the rapidity with which cockroaches immobilized at 15° C. reverse the toxic effects of the toxicant when transferred to 35° C.

Since the rate of penetration of pyrethrum was accelerated at a higher temperature, it was not surprising that a high level of pyrethrum was present internally, as evidenced by the rapid onset of symptoms when treated cockroaches were transferred to a lower temperature. The mechanism required to keep the concentration of the toxicant reduced at sites critical to its action is probably one of the paramount regulators of a relative insensitivity to pyrethrum at high temperatures.

A study of the blood of pyrethrum-treated cockroaches provided another method of examining the negative temperature coefficient of pyrethrum. A preliminary examination of the haemolymph of treated insects demonstrated that the blood would produce a paralytic reaction in sarcophagid flies provided that the cockroaches from which the blood was obtained were exhibiting symptoms of poisoning. Using small doses of pyrethrum, it was demonstrated that the toxicity of the cockroach blood to flies could be correlated with the stage of poisoning exhibited by the cockroach (table 5). Considering excitation, uncoordination and prostration as sequential symptoms of toxic reactions of pyrethrum, the blood was shown to become increasingly toxic as one stage evolved into another towards complete immobility. Consequently, the duration of paralysis of injected flies could be qualitatively correlated with the stage of poisoning of the cockroach. On the other hand, if cockroaches were treated with a high concentration of pyrethrum, paralysis occurred almost immediately. Blood removed several hours after onset of complete paralysis was of the same relative toxicity as that removed immediately after prostration occurred, indicating that the concentration of toxic material in the blood was fairly constant after complete paralysis occurred. If the toxic principle in the blood is pyrethrum, active processes such as detoxication or storage must be occurring to produce a relatively constant concentration of the insecticide present in the haemolymph while penetration is occurring.

Table 5.—Average duration of paralysis of sarcophagid flies injected with 6 μl. of blood from pyrethrum-treated cockroaches at various stages of poisoning (26–27° C.).

Condition of Cockroaches	Number of Flies Injected	Average Time Prostrate at 35° C. After Transfer (Min.)
Excited	10	7
Uncoordinated	10	12
Prostrate	10	21
Normal	10	0

Table 6.—Duration of paralysis of sarcophagid flies injected with 3 μl. of blood from C^{14}-pyrethrum-treated cockroaches and an evaluation of the blood samples for radioactivity.

Sample	Number of Flies Injected	Average Duration of Paralysis (Min.)	Radioactivity (Cpm.)
No. 1	10	21	0
No. 2	10	26	0
No. 3	10	20	0

The absence of radioactivity in the blood of cockroaches treated with C^{14}-pyrethrum indicates that the toxin is not pyrethrum. As the data in table 6 indicate, although the different samples were capable of paralyzing flesh flies, no evidence of radioactivity was found. It was impossible that metabolites of pyrethrum were present which were non-radioactive, since the insecticide was biosynthesized and was randomly labelled. The possibility that the concentration of pyrethrum was below the sensitivity of the radioactive assay method is unlikely. The concentration of pyrethrum would be so very low that the blood would have probably negligible bioassay toxicity. Furthermore, these findings are supported by the fact that as little as 0.1 μg of C^{14}-pyrethrum can be nearly quantitatively extracted from normal cockroach blood to which it has been added. It is therefore evident that part of the toxic action of pyrethrum is due to its stimulating the production of a toxic substance in the cockroach.

The effect of temperature on this blood-carried toxin is quite striking. Blood removed from treated cockroaches held at 35° C. was completely non-toxic to sarcophagid flies. On the other hand, blood removed from treated cockroaches held at 15° C. contained the toxin (table 7). Like DDT-poisoned cockroaches, the presence of the toxin is temperature-dependent provided that the concentration of applied insecticide is not too high. This fact is further emphasized by the observation that the blood collected from cockroaches which had been successively alternated between 35° C. and 15° C. was always toxic at the lower temperature and non-toxic at the elevated one.

The effect of pyrethrum synergists on the ability of the cockroach to tolerate pyrethrum at higher temperatures was investigated using the presence or absence of a toxin in the haemolymph as a criterion (bio-assayed on flesh flies). Chamberlain (1950) demonstrated that house flies are more susceptible to pyrethrum at higher temperatures when a synergist is applied simultaneously,

Table 7.—Duration of paralysis of sarcophagid flies injected with 6 μl. of blood from pyrethrum-treated cockroaches at 15° C. and 35° C.

Temperature (° C.)	Condition of Cockroaches	Average Duration of Paralysis (Min.)
15	Prostrate	24
35	Normal	0

Table 8.—Duration of paralysis of sarcophagid flies injected with 6 μl. of blood from cockroaches treated with pyrethrum (1 μg.) with and without piperonyl butoxide (10 μg.) and held at 35° C.

TREATMENT	NUMBER OF OF FLIES INJECTED	AVERGAE DURATION OF PARALYSIS (MIN.)
Pyrethrum	10	0
Pyrethrum+piperonyl butoxide	10	10
Piperonyl butoxide	10	0

a fact which has also been found to be true with the American cockroach. Furthermore, the inability of the cockroach to tolerate pyrethrum at 35° C. in the presence of a synergist was correlated also with the presence of a toxic principle in the haemolymph (table 8). Assay of the haemolymph for piperonyl butoxide (Blum 1955), the synergist employed in this study, demonstrated the absence of this material. Analysis of cockroach tissues demonstrated the presence of the synergist in the fat body and central nerve cord.

The concentration of the toxic agent in blood removed from pyrethrum-poisoned cockroaches diminished with time (as indicated by bio-assay) indicating the instability of the material or its degradative metabolism by components of the blood (table 9).

SUMMARY.—In the American cockroach, topically applied pyrethrum shows a negative temperature coefficient of action between 15° C. and 35° C. The 24-hour LD-50 for pyrethrum is about 1 μg. at 15° C. and about 6 μg. at 35° C.

Employing C^{14}-pyrethrum, it has been determined that at 35° C. the rate of penetration of pyrethrum is more than twice that which occurred at 15° C. Cockroaches prostrate at 15° C. can be returned to normalcy by transferring them to 35° C., a process which can be repeated for several hours. Since cockroaches transferred from 35° C. to 15° C. became prostrate more rapidly than those held continuously at 15° C., the insecticide (or some metabolic toxin) was probably in the vicinity of its site of action at 35° C. but was ineffective.

Pyrethrum poisoning in the cockroach was associated with the presence of a blood-carried toxin which did not manifest its toxicity at 35° C. Employing C^{14}-pyrethrum, it has been determined that the toxic blood from pyrethrum-poisoned cockroaches was non-radioactive, indicating that the toxic material in the blood was not pyrethrum. The symptoms of poisoning manifested by pyrethrum-treated cockroaches were correlated with the concentration of toxin as determined by bio-assay. Piperonyl butoxide increased the susceptibility of the cockroach to pyrethrum at higher temperatures, as indicated by observations, and the existence of the toxin at higher temperatures. The toxic agent in blood lost its activity when stored at room temperature.

Table 9.—Duration of paralysis of sarcophagid flies injected with blood removed from pyrethrum-treated cockroaches and held at room temperature (26–27° C.) for different intervals of time.

BLOOD HELD AT ROOM TEMPERATURE (MIN.)	NUMBER OF FLIES INJECTED	AVERAGE DURATION OF PARALYSIS (MIN.)
0	10	26
30	10	19
60	10	12
180	10	3

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Resistance of the Codling Moth to DDT Sprays[1]

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Rumors that the codling moth (*Carpocapsa pomonella* (L.)) might be becoming resistant to DDT started in 1952, seven years after it was first applied to large orchard plots, and have continued. Failures by growers to maintain control with DDT have been reported from several states. Cutright (1954) reported that in one southern Ohio orchard this insecticide had not been effective since 1952. According to E. J. Newcomer, in 1953 growers near Gleed, Washington, failed to obtain satisfactory results. Workers at the Vincennes, Indiana, fruit-insects laboratory observed a similar condition in an orchard near Henderson, Kentucky, where DDT had maintained good control since 1945.

These observations prompted work at Vincennes to determine the effectiveness of DDT sprays on different strains of codling moth larvae.[2] Progeny from 16 strains were studied during 1953, 1954, and 1955. Three strains originated in Kentucky, one in southern Illinois, five in Indiana, five in the Yakima Valley of Washington, one near Hamilton, Ohio, and one was a laboratory-reared strain from Riverside, California. Coming from orchards where DDT has been heavily or indifferently applied, or used for only one season or not at all, these strains are believed to be representative of those likely to be present in two- and three-brood areas throughout the country.

Stocks of these strains, except the one from California, were obtained from corrugated-strawboard bands placed on infested trees. About January following collection they were placed in cold storage at 35° F. and held there until used. Larvae and pupae of the California strain were received a few weeks before they were needed and held in a refrigerator until used. Laboratory procedures followed in rearing the larvae and infesting apples were patterned after those described by Steiner (1949), with modifications to fit the conditions of these tests. Overwintering larvae, generally 500 to 600 per strain, were removed from storage at intervals for rearing to provide newly hatched larvae for use in July and August. Within 30 minutes after hatching, larvae were transferred in pairs, one to a DDT-sprayed apple and one to an unsprayed apple. Sixty apples and five larvae per apple were used per test of each strain, except strains 10 and 16, of which not enough larvae were available. The intention was to transfer the larvae of all strains in a given experiment to apples at about the same time, but this was not always possible for the same reason.

In 1953 and 1954 the DDT sprays were applied to orchard trees, and the fruit was picked and taken to the laboratory for exposure to the test strains. In 1955 the fruit was sprayed while being revolved on a turntable in the laboratory and then exposed to the test strains as previously. All the sprays were applied with a high-pressure hydraulic sprayer. The trees bearing fruit for the tests were protected from insect injury with parathion to within 6 to 10 weeks of the time of the DDT application. Chemical analyses showed less than 0.02 mg. of parathion per square centimeter of fruit surface at the start of the experiment. The success of the larvae in entering the check fruit indicated that the parathion residue did not affect the results of the experiments.

Three midwestern strains of larvae collected in the fall of 1952 were used in the 1953 tests. The apples were sprayed with ¾ pound of 50% DDT wettable powder in 100 gallons of water. The DDT deposits averaged 3.2 micrograms per square centimeter immediately after application and 2.8 micrograms a week later.[3] These amounts are considerably below the 7.5 micrograms considered necessary for good codling moth control under midwestern conditions (Hamilton *et al.* 1954), but it was expected that they would give a degree of control that would accentuate any differences in the susceptibility of the strains to DDT. As shown in table 1, all strains were readily killed by the fresh deposits, and differences resulting from exposure to the weathered deposits were not in relation to their history of exposure to DDT. However, there appeared to be a real difference in the vigor of the strains as measured by their ability to enter unsprayed apples.

Table 1.—Reduction of entrances by different strains of codling moth larvae in fruit sprayed with ¾ pound of 50% DDT wettable powder per 100 gallons, 1953.

Strain	Source	History of DDT Use	Per Cent of Entrances in Unsprayed Apples	Per Cent Reduction from—	
				Fresh Deposits (3.2)[a]	7-Day-Old Deposits (2.8)
1	Henderson, Ky.	Heavy since 1945 poor control in 1952	71	95.9	81.8
2	Omaha, Ill.	Moderate since 1947	78	94.3	69.9
3	Odon, Ind.	None	46	96.8	88.5

[a] Figures in parenthesis indicate DDT deposit in micrograms per square centimeter.

In 1954 seven strains of codling moth were included in the tests. Following the same methods as used in 1953, several series of tests were made with fresh and weathered deposits from various dosages of DDT. These deposits ranged from 3 to 11 micrograms per square centimeter. Table 2 shows that strain 4, from Gleed, was the most successful in entering sprayed fruit, particularly when 1 pound or less of the wettable powder was used. DDT deposits of 10 to 11 micrograms per square centimeter, such as result from the 2-pound application, controlled the Gleed strain about as well as they did the others. The other strains showed no resistance to DDT, although strain 8 readily entered fruit on which the deposits had

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[2] W. D. Armstrong, of Kentucky Agricultural Experiment Station, provided the larvae of strain 13; Martin Barnes, of California Agricultural Experiment Station, those of strain 10, a laboratory strain; C. R. Cutright, of Ohio Agricultural Experiment Station, those of strain 16; and E. J. Newcomer, of the Yakima, Wash., laboratory of the Entomology Research Branch, those of strains 4, 5, 6, 11, and 12. The remaining strains were collected by the staff of the Branch laboratory at Vincennes, Ind.

[3] All residue analyses were made by J. E. Fahey, of the Vincennes laboratory.

Table 2.—Per cent reduction in entrances by different strains of codling moth larvae in fruit sprayed with different dosages of 50% DDT wettable powder per 100 gallons, 1954.

Strain	Source	History of DDT Use	Fresh Deposits				Weathered Deposits	
			½ lb. (3.0)[a]	¾ Lb. (4.8)	1 Lb. (4.1)	2 Lb. (10.7)	¾ Lb. (3.1)	2 Lb. (7.0)
4	Gleed	Several years, 6 applications in 1953. Poor control in 1953	70.2	37.2	64.8	87.6	4.9	54.9
5	Yakima A	Various insecticides in experimental orchard	—	96.9	—	97.3	88.2	100
6	Yakima B	Poorly sprayed several years	—	100	—	—	96.3	—
7	Vincennes A	Lightly sprayed several years	—	97.8	—	—	87.3	—
8	Vincennes B	Moderately sprayed since 1946	94.2	98.3	97.1	100	33.3	85.8
9	Carlisle, Ind.	Lightly sprayed several years	98.2	100	98.2	100	86.8	98.0
10	Riverside, Calif.	Never exposed to DDT	—	—	—	97.9	78.3	—

[a] Figures in parenthesis indicate DDT deposit in micrograms per square centimeter.

weathered to 3.1 micrograms per square centimeter. However, fresh deposits of this magnitude controlled this strain.

Nine strains of larvae were tested in 1955—strains 1, 4, and 8 that had been used in previous years, and six new ones, two from Washington, two from Kentucky, and one each from Ohio and Indiana. Three series of tests were conducted, one each in which the larvae were exposed to fresh deposits resulting from application of three strengths of DDT. Three of the nine strains were definitely resistant to DDT, strains 4 and 11 from Washington, and strain 16 from Ohio (table 3). The Ohio strain had been found resistant by Cutright (1954). As previously, differences in susceptibility of codling moth larvae from midwestern orchards were of no significance.

In 1955 limited tests were made to compare the susceptibility to DDT of adults of known resistant and nonresistant larval strains. Adults of nonresistant strains 1, 12, 14, and 15 and of resistant strains 4 and 16 (table 3) were exposed for 1 hour to surfaces dipped in a DDT spray. Although the numbers of moths available were too small to give significant results, the indications were that strains resistant as larvae may also be resistant as adults. For example, within 24 hours after exposure to DDT, 75% of the adults of all the nonresistant strains were dead as compared with only 25% of the adults of the resistant strains.

Favorable weather for codling moth activity, poor spray coverage, and, in some instances, the use of adjuvants or other pesticides that affect adversely the amount and persistence of DDT deposits were the principal causes of the increase in damage in midwestern orchards in 1953 (Hamilton *et al.* 1954). Although these factors apparently are responsible for much of the damage that continues to be reported, in general it is becoming more difficult to obtain good control with this insecticide in southern Indiana, southern Illinois, and Kentucky. This suggests that at least some resistance is present. Data obtained from one orchard show that in 1943 the use of DDT beginning with the seventh cover spray on July 22 completely stopped codling moth entries, but in 1950 worm holes averaged 0.3 per 100 apples after seven applications and in 1954 71.1 after nine applications.

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Table 3.—Per cent reduction in entrances by codling moth larvae of different strains in fruit sprayed with different dosages of 50% DDT wettable powder per 100 gallons, 1955.

Strain	Source	History of DDT Use	Control	½ Lb. (3.8)[a]	1 Lb. (5.0)	2 Lb. (8.4)
4	Gleed, Wash.	Several years	Poor in 1953	14.8	45.4	68.5
11	Yakima, Wash., C	Several years	Poor in 1954	21.0	16.7	53.6
12	Yakima, D	None used	—	94.9	97.3	100
1	Henderson, Ky.	Heavy since 1945	Poor since 1952	96.2	100	100
13	Paducah, Ky.	Used since 1946	Poor in 1954	97.2	100	100
14	Benton, Ky.	Used 1947 only	—	96.2	100	100
8	Vincennes, Ind., B	Moderate since 1946	—	84.6	97.7	100
15	Vincennes, C	Poorly applied since 1947	—	88.3	96.8	99.1
16	Hamilton, Ohio	Used since 1946	—	—	20.0	76.3

[a] Figures in parenthesis indicate DDT deposit micrograms in per square centimeter.

Tests with New Insecticides to Control Green June Beetle Larvae in Tobacco-Plant Beds[1]

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The green June beetle, *Cotinis nitida* (L.), is the most important insect enemy of tobacco seedlings in plant beds in north-central Tennessee. The larvae feed on organic matter found in the rich soils used for the production of tobacco plants. In searching for food they disturb the top soil and uproot the seedlings. The heaviest infestations occur in rich, moist soil to which manure or other organic matter has been added. Heavy applications of manure incident to an expansion of the beef-cattle industry in this area caused the infestation of these larvae to increase steadily from 1936 to 1952. In July and August 1952, and again in 1953, severe drouth interfered with the establishment of newly hatched larvae and greatly reduced the infestation. No larvae could be found in the vicinity of Clarksville in 1954 or 1955.

A bran bait containing paris green, recommended by McKinney & Milam (1926), was for many years the accepted means of control. The bait was moderately effective, but acted too slowly to provide satisfactory control of heavy infestations. Jewett (1943) recommended apple refuse, wheat bran, and cornmeal as bases for paris green bait. Scott & Milam (1944) reported that the larvae could be controlled with gasoline applied as a soil fumigant. Allen & Pollard (1947) demonstrated that a bait made of wheat middlings and barium, potassium, or sodium fluosilicate was more effective than bran combined with paris green.

This paper reports the results of experiments conducted at Clarksville, Tenn., during the period 1945 to 1953 with several of the new insecticides in baits, dusts, and drenches to determine their effectiveness against the larvae in tobacco plant beds.

Methods and Materials.—The experimental plots, except as otherwise indicated, measured 5 by 10 feet. They were prepared in the same manner as plant beds are prepared except that the soil was not sterilized. It was worked to a depth of 6 inches with the usual farm equipment. The plots, arranged in Latin squares, were separated by 6-inch boards imbedded 2 inches in the soil. Unless otherwise indicated, there were 6 replicates of each treatment. The treated areas were covered with tobacco cloth to exclude birds, chickens, and other animals. At frequent intervals during 4 to 7 days following the treatments, dead larvae were collected from the surface of the plots. At the end of this time most of the plots were excavated to a depth of 12 inches to determine the numbers of dead and living larvae in the soil. In plots that were not excavated the dead larvae found on the soil surface and the tunnel entrances made by larvae were counted. All moribund larvae were counted as dead, as it is easily demonstrated that they do not re-enter the soil.

The dusts were applied by means of a cloth sack, parathion at the rate of 4.1 pounds and the others at 1.5 pounds per 100 square yards. Baits, slightly moistened, were broadcast by hand at 12 to 18 pounds per 100 square yards. Drenches were applied from a sprinkling can at the rate of 54 gallons per 100 square yards. Fumigants were poured into holes, which were immediately covered with moist clay or small pieces of sod. D-D mixture was applied undiluted in twenty-four 6-inch holes per square yard. Gasoline was poured into 8-inch holes, 1 foot apart, at the rate of 14 gallons per 100 square yards.

Results.—The results of these tests are summarized in tables 1 and 2. Since about 10% of the larvae do not come to the surface and are not responsible for any injury to tobacco seedlings, the tunnel entrances formed in the plots that were not excavated probably provided a more reliable indication of the effectiveness of the treatments than the dead and living larvae found below the surface of the excavated plots. Many of the larvae that remain below the surface are killed by fumigants, but baits, drenches, and dusts are ineffective against them. Of the total treatments tested, 22 were less than 40% effective. The data for these treatments are not shown in the tables. Among the insecticides eliminated were sabadilla, BHC, ryania, methoxychlor, and tetraisopropyl pyrophosphate. It is believed that most of the dead larvae found in the check plots had entered from adjacent treated plots.

Table 1.—Mortality of green June beetle larvae in tobacco plant beds treated with insecticides applied to the soil.

Treatment	Toxicant per 100 Square Yards	Total Larvae Found in Plots	Per Cent Mortality
Experiment 1—1945			
Baits, wheat middlings:			
Barium fluosilicate, 14%	40.0 oz.	301	51
DDT, 1%, +cottonseed oil, 10%	2.0 oz.	466	52
Fumigants:			
D-D mixture	0.5 gal.	255	59
Gasoline	14.0 gal.	258	67
Checks	—	306	0
Experiment 2—1947			
D-D mixture	1.0 gal.	60	46
Gasoline	14.0 gal.	73	56
Checks	—	41	0
Experiment 3—1948			
Bait, wheat middlings and bran (1:1):			
Parathion, 0.4%	1.1 oz.	805	97
Barium fluorisilicate, 13%	25.0 oz.	489	59
Fumigant: Gasoline	14.0 gal.	485	73
Drench: DDT, emulsion, 0.4%	28.0 oz.	758	97
Check	—	327	0
Experiment 4—1949			
Dust: Parathion, 1%	0.7 oz.	158	60
Drench: Parathion, 0.9 oz. 25% WP in 18 gal.	0.7 oz.	175	46
Checks	—	271	0
Experiment 5—1950			
Drench: Parathion, 7.8 oz. 10% WP in 18 gal.	2.3 oz.	402	49
Checks	—	414	0
Experiment 6[a]—1953			
Dusts: Lindane, 5%	1.2 oz.	70	66
Parathion, 1%	0.2 oz.	72	90
Malathion, 5%	1.2 oz.	52	67
Endrin, 1%	0.2 oz.	54	70
Checks	—	39	15

[a] Each treatment applied to five 15-square-foot plots.

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Table 2.—Reduction in tunnels made by green June beetle larvae in tobacco plant beds treated with insecticides in dusts and drenches. Clarksville, Tenn., 1951–1952.

TREATMENT	OUNCES OF TOXICANT PER 100 SQUARE YARDS	TOTAL LARVAE FOUND IN PLOTS	NEW TUNNELS	PER CENT REDUCTION IN NEW TUNNELS
	Experiment 1—1951			
Dusts: Parathion, 1%	0.2	346	223	69
Toxaphene, 20%	4.8	79	421	42
Aldrin, 2%	0.5	61	421	42
Dieldrin, 2%	0.5	131	361	50
Chlordane, 10%	2.4	61	438	40
Checks	—	27	728	—
	Experiment 2—1951			
Drenches: Parathion, 24 oz. 25% WP in 18 gal.	18.0	207	10	92
Malathion, 24 oz. 25% WP in 18 gal.	18.0	124	48	60
Dusts: TDE 10%	2.4	181	53	55
Dilan, 5%	1.2	194	55	54
Lindane, 10%	2.4	263	31	74
Checks	—	48	119	—
	Experiment 3[a]—1952			
Drenches: Parathion, 3 lb. 25% WP in 18 gal.	36.0	98	17	85
Malathion, 6 lb. 25% WP in 18 gal.	72.0	31	23	80
Dusts: Parathion, 1%	0.2	85	33	71
Aldrin, 4%	1.0	10	68	40
Checks	—	0	113	—

[a] Each treatment applied to four 18-square-foot plots.

With the possible exception of 10% chlordane dust, all the treatments were found to be safe for application to tobacco plant beds. Plants treated with chlordane were slightly stunted. The DDT emulsion drench that contained 0.2% of sodium lauryl sulfate and 4% cottonseed oil caused the leaves of seedlings to become oily, but this condition disappeared within a few days, and the treated plants developed normally.

The larvae are extremely active when night temperatures do not fall below 60° F., but at lower temperatures they tend to remain in the soil and therefore do not come in contact with insecticides on the soil surface. However, all surface treatments retained their effectiveness for several days, and many larvae were killed after a week or more.

Regardless of the amount applied or the method of application, parathion was quickly effective as measured by surface activity of the larvae. Parathion at 1.5 pounds per 100 square yards in a 1% dust was quickly effective when temperatures remained above 60° F. A bait containing 0.4% of parathion was more effective than drenches and dusts containing this material.

Barium fluosilicate baits were moderately effective, especially on warm nights, when large numbers of larvae came to the surface to feed. Malathion was not available until 1951, and was therefore not tested as adequately as parathion. Applied in a drench at the rate of approximately 18 ounces per 100 square yards, this insecticide reduced the formation of new tunnels 60%. A similar application of parathion caused a reduction of 92%. When the dosage of malathion was increased to 72 ounces per 100 square yards, the formation of new tunnels was reduced 80%. A 5% malathion dust at 1.5 pounds per 100 square yards reduced the larval infestation 67% as compared with 90% by a 1% parathion dust.

Gasoline at 14 gallons per 100 square yards was effective but not equal to parathion. DDT-cottonseed oil emulsion was one of the most effective treatments tested, but DDT dusts and suspension drenches were unsatisfactory. D-D mixture at 0.5 and 1 gallon per 100 square yards was less effective than parathion, DDT emulsion drench, or gasoline. Ten % chlordane dust at 1.5 pounds reduced the formation of new tunnels 40%. Lindane dust at 1.2 ounces reduced the larval infestation 66%. When the dosage was increased to 2.4 ounces, the reduction was 74%. A single test with 1% endrin dust indicated that this material applied at 1.5 pounds warranted additional tests. Dusts containing 20% of toxaphene, 2% of aldrin or dieldrin, or 10% of TDE, applied at 1.5 pounds per 100 square yards, were less effective than similar applications of 1% parathion dust.

SUMMARY.—Several insecticides were tested in baits, dusts, and drenches for the control of larvae of the green June beetle, *Cotinis nitida* (L.), in tobacco plant beds at Clarksville, Tenn., during the period 1945–1953.

Parathion was the most effective insecticide tested. A 1% parathion dust at 1.5 pounds per 100 square yards provided satisfactory control when night temperatures remained above 60° F. Similar results were obtained with baits and drenches containing parathion. Barium fluosilicate baits were moderately effective. Malathion, gasoline, DDT, D-D mixture, chlordane, lindane, endrin, and TDE were toxic to the larvae but less effective than parathion. Sabadilla, BHC, ryania, methoxychlor, and tetraisopropyl pyrophosphate were only slightly effective.

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Biology Studies of the Pickleworm[1]

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The pickleworm (*Diaphania nitidalis* (Stoll)) limits the production of cucurbit crops during the summer and fall months in the southeastern part of the United States. In southern portions of Texas and Florida it also is a pest during the winter and spring. The insect frequently causes considerable damage in adjoining States and occasionally occurs in more northern States in late summer and early fall. Quaintance (1899, 1901) described the insect and its habits as a serious pest in Georgia and included a complete bibliography to that time. Smith (1910, 1911) published similar information regarding the insect in North Carolina, and included a bibliography through 1910. Fulton (1947) gave additional information regarding the habits of the pickleworm in North Carolina. More recently, Dupree *et al.* (1955) reported results of their studies of the insect in Georgia.

In the spring of 1948 a study was begun at Charleston, S. C., of those phases of the habits of the pickleworm that may have a bearing on its control. This study was continued through 1954. A few notes on the seasonal occurrence and overwintering were made by the senior author prior to 1948. Results of the control studies through 1951 have been published (Reid & Cuthbert 1953). This article presents the information obtained on the biology of the insect.

Distribution.—The pickleworm has been reported from all States east of the Mississippi River with the exception of Massachusetts, Vermont, New Hampshire, and Maine. It has also been reported from Texas, Kansas, Nebraska, Iowa, Missouri, Arkansas, and Louisiana. Various other reports state that the species also occurs in Canada, Mexico, Central America, South America, and the West Indies. In the northern portion of its range in the United States the pickleworm occurs in destructive numbers only during occasional years. In the south Atlantic and Gulf Coast States it is a serious pest practically every year.

Description and Habits.—*Adults.*—Adults of the pickleworm are rather pretty moths that are easily distinguished from other insects, with the possible exception of the melonworm. Pickleworm moths are about $\frac{5}{8}$ inch long, exclusive of the antennae, and have a wing spread of $1\frac{1}{8}$ to $1\frac{1}{4}$ inches. The body and wings, viewed from above, are yellowish brown with an iridescent purplish reflection. Tips of the abdomen bear a brushlike appendage consisting of flattened, curved scales that are yellowish at the base and light brown at the tip. This brush is smaller and the scales more curved in females. The fact that the brush can be partially withdrawn makes this difference between the sexes less apparent at times. The moths frequently can be seen holding their brushes above the body level and slowly waving them in a circular fashion.

The adults are active and lay their eggs only at night. None have been seen in the fields during the day, except a few that appeared to be affected by insecticides and could not fly well. Adults were found in squash plantings shortly after dark. They were not strongly attracted by a flashlight, and very few were taken in electric light-traps operated near infested plantings. All adults liberated in cucumber fields or elsewhere outdoors flew at once towards nearby woods or hedgerows, usually flying with the wind and as high as 50 feet from the ground. Several were seen to alight in trees. Moths placed on the underside of cucumber leaves or on other plants in the field and in the woods during the day stayed there for several hours. It appears that they spend daylight hours in vegetation near cultivated fields, possibly on trees and high bushes.

Numerous attempts were made to secure fertile eggs from moths. Cages of various sizes and shapes, including a large outdoor screen house about 10 by 35 by 80 feet, were used. Egg white, flowers of squash, cucumber, broccoli, mustard, lantana, abelia, goldenrod, honeysuckle, ageratum, crotalaria, and clover, and solutions of honey, yeast hydrolysate, and sugar were tried as food for the adults. Water was supplied, and potted squash or cucumber plants were kept in the cages. Individual pairs and varying numbers of both sexes were caged together. The moths were observed to feed sparingly on lantana and abelia flowers. Only a few eggs, none of which were fertile, were obtained. Most of the caged specimens died within a week; none lived more than 9 days.

Eggs.—Eggs of pickleworm moths are small (about 0.4 to 0.7 mm.) and irregular in shape, have thin coverings, and are white to yellowish. They are laid singly or in clusters of two or more among the hairs on the flower and leaf buds, or the tender leaves, on the small fruit, or on the stalks and vines. The eggs are not easily seen with the naked eye. As no fertile eggs were laid by caged adults, complete oviposition and incubation data were not obtained. All field-collected eggs hatched within 3 days.

Larvae.—Newly hatched pickleworms are about $\frac{1}{16}$ inch long, have a head that is wider than the body, and are whitish except for a black spot on each side of the head and dark brown tubercles on the body segments. These tubercles, which occur in transverse rows, become more pronounced and the surrounding corneous areas become darker as the larvae mature. They give the appearance of black spots in the third and fourth instars, but in the fifth instar the spots are lost and the larvae become yellowish green to whitish, depending upon the food being eaten. Full-grown larvae are about $\frac{5}{8}$ to $\frac{7}{8}$ inch long, and have brown heads.

From 2 to 4 days before pupating pickleworms stop feeding and move to one of the leaves of the plants, or sometimes to leaves or trash under the plant. They spin a thin, loose web of silk around the body, folding the leaf partly around themselves. They then become reddish brown in color and shorter and thicker in shape. About

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1 day before pupating the larvae again become yellowish green to whitish.

Caged first- or second-instar larvae required 8 to 10 days to complete their development (indoors) during May and June, 9 to 15 days (outdoors) during July and August, 20 days (outdoors) in August and September, and 25 to 28 days (outdoors) in October and November. Larvae reared from eggs collected in the field required 8 to 10 days to mature in an indoor cage in August and 12 days in September. From 2 to 4 days were spent in the inactive prepupal stage. This means that the larvae required at least 10 to 25 days to develop in the field. During September and October, the period of their greatest damage to commercial plantings in the Charleston area, individual pickleworms fed 2 to 3 weeks. When supplied with an abundance of food in an indoor cage where temperatures were more nearly constant, they completed their development in 6 or 7 days in midsummer.

Pupae.—Pickleworm pupae are found in a thin web in a folded large leaf on or under the plants. Sometimes pupae occur among trash on the ground beneath the plants. Occasionally a pupa has been found hanging loosely from the underside of a leaf. Newly formed pupae are greenish white. Older ones are shiny reddish brown or copper-colored. The anterior end of the pupa is somewhat blunt; the posterior end is pointed and bears several spines which have curved tips that hold the pupa in its thin cocoon. Pupae are $\frac{1}{2}$ to $\frac{3}{4}$ inch long. In field cages the pupal stage lasted from 6 to 13 days from May until August 1948, at mean temperatures between 72.5° and 81° F., and 11 to 31 days from September until mid-December, at mean temperatures between 59° and 68° F. No record was made of the pupal period of specimens kept in hibernation cages, but some of them remained in that stage for at least 70 days between October and mid-January. The pupal period of pickleworms reared in an office building ranged from 6 to 11 days during the summer and from 10 to 22 days in the fall and early winter.

Length of Life Cycle.—In field cages the life cycle, less the preoviposition period of the adult, lasted from 22 to 28 days between May and August 1948, at mean temperatures between 75° and 77° F., and from 37 to 53 days between mid-August and mid-December, at mean temperatures of between 59° and 68° F. Specimens caged indoors during June, July, and August lived 18 to 22 days.

SEASONAL OCCURRENCE.—Periodic (weekly or more frequent) examinations of squash blooms beginning when the first ones opened, usually late in April, disclosed the following initial dates of larval occurrence at Charleston: May 5 in 1948, May 20 in 1949, May 10 in 1950, May 18 in 1951, May 30 in 1952, May 20 in 1953, and June 11 in 1954. Less complete notes show that the first specimens were found in 1946 on May 29 and in 1947 on May 19. The first pickleworm brood is too small to cause appreciable loss to early-spring cucurbits in the vicinity of Charleston. Occasionally the second brood occurs early enough in June to cause slight damage near the end of the harvest period. During the summer and fall the insect slowly increases in abundance with considerable overlapping of broods. Any untreated host plants growing during midsummer and fall are often heavily infested and may be killed. Pickleworms remain active until their host plants are killed by frost.

Quaintance (1901) reported that June 17 was the earliest date that he found the pickleworm at Experiment, Ga. Smith (1911) and Fulton (1947) did not find specimens before July 6 in the vicinity of Raleigh, N. C. Sullivan (1924) stated that the insect is not ordinarily a serious pest in Missouri, but that in 1923 it caused severe damage late in July to cantaloups retarded by adverse weather. According to Watts (1939), pickleworm adults became active around June 1 to 5, 1939, and half-grown larvae were first found on June 15 near Blackville, S. C. This was said to have been unusually early. Dupree *et al.* (1955) reported May 18 as being the earliest seasonal record in Spalding County, Ga.

According to reports of several entomological workers,[2] the pickleworm is active on native and cultivated hosts throughout the winter in southernmost Florida, it occurs on cultivated hosts in central and southwest Florida in March and early April, and in south Georgia in late April. An apparently unpublished report by D. K. McMillan, dated December 31, 1908, indicated that the insect feeds throughout the winter in the vicinity of McAllen, Tex., wherever remnants of cucurbit crops exist. The authors found a few pickleworms in an abandoned squash field near Tampa, Fla., on December 16, 1949. The adults emerged during the following January.

In Cooperative Economic Insect Reports published by the U. S. Department of Agriculture between 1950 and 1953, pickleworm infestations were reported in Florida throughout the year, in South Carolina from May to November, in Georgia from June to November, in Alabama, Mississippi, and Wisconsin during July, in Delaware during August, in Tennessee and North Carolina during August and September, in Virginia from August to October, and in Maryland in September.

In correspondence dated April 2, 1954, W. H. Enne, of the University of Missouri, stated that the pickleworm appeared unusually early (June) and was universally destructive to cucurbits in that State in 1950. This report and the July report from Wisconsin in 1953 are unusually early records for such northern areas.

Several early writers, including Quaintance (1899) in Georgia and Smith (1911) in North Carolina, stated that the pickleworm passes the winter as a pupa. However, no one apparently obtained adults from overwintered pupae. Fulton (1947) concluded that the insect does not pass the winter in North Carolina or northward and that it survives only in areas where living host plants occur during the greater part of the winter. Dupree *et al.* (1955) reached similar conclusions in their studies in Georgia.

Our studies indicate that the pickleworm does not live throughout the winter in the Charleston area. No specimens of any stage have been seen in fields or woods in midwinter and early spring. The species appears there late in the spring. No individuals passed the winter in a total of 23 hibernation cages in 1931 and 1948–52. These screen-wire cages were 3 by 4 feet and from 1 to 3 feet

[2] Unpublished correspondence with J. W. Wilson, E. G. Kelsheimer, and W. G. Genung of the Florida Agricultural Experiment Station and F. S. Chamberlin of the Entomology Research Branch.

high. From 1 to 7 bushels of pickleworm-infested squash or cucumbers were placed in each in October and November. The cages were kept in locations that afforded different exposures. Some were in the woods, some in the open, and others in protected places behind buildings. A total of 1,353 pickleworm moths emerged in these cages, most of them in November and December. These moths were unable to fly when the air temperature was less than 50° F. and all died within a week. No moths emerged after January 20.

It now appears that the pickleworm does not overwinter in Georgia or the Carolinas. It feeds throughout the winter in southern Florida, and possibly other semitropical areas, and evidently gradually moves northward each spring and summer.

INJURY CAUSED BY PICKLEWORMS.—Pickleworms of the first three instars feed on the parts of the plants where the eggs were laid. Flower and leaf buds are their favorite food. On squash the large staminate flowers are often the first portion attacked. They can complete their larval development in these large flowers. The inner portions of flower buds and the more mature flowers often are destroyed, with the result that the pistillate ones produce no fruit. The fruits, stalks, and vines are tunneled by the larvae, especially the last two instars. The growing tips of the plants may be destroyed, or the entire plant killed. Fruits that are entered are made unfit for human use because of the tunneling and excrement of the insect. Infested fruits sometimes decay. Infested cucumber fruits, those about 1 inch or less in length, make little or no further growth and finally dry up. Fruits entered after they are half grown continue to develop, but are malformed at harvest time. Fruits tunneled nearer maturity are not as likely to be deformed.

Pickleworms often spin thin silken webs over their entrance holes. This habit evidently renders them less susceptible to attack by predaceous or parasitic insects.

HOST PLANTS.—Cucurbits appear to be the only plants attacked by the pickleworm. Some are much more attractive than others. The summer squashes are usually the first crops to be infested and apparently are the preferred hosts. Cucumbers and cantaloups also are readily attacked. The first pickleworms found in the spring of 1949 were in cucumbers in spite of the fact that there were several nearby fields of squash. The young tender fruits of winter squash and pumpkins were also injured. Pickleworms were found in the blooms of a white-flowered gourd, *Lagenaria siceraria*, and in the fruit of a native creeping cucumber, *Melothria pendula*, at Charleston, S. C., but these plants are apparently not preferred hosts. The insect was found only once on watermelons; other workers have reported an occasional infestation of this crop.

INSECTS THAT MIGHT BE MISTAKEN FOR PICKLEWORMS.—*Melonworm.*—The melonworm (*Diaphania hyalinata* (L.)) and the pickleworm are close relatives, often are found together in the field, and sometimes are not distinguished. Eggs, full-grown larvae, and pupae of the two species are similar in appearance. Adults and immature larvae can be identified upon close examination. The abdomen and inner portions of the wings of melonworm moths are silvery or pearly white, whereas inner portions of wings of pickleworm moths are light yellow and the upper surfaces of the abdomen yellowish brown. Melonworm moths remain in the field during the daytime and when disturbed can be seen flitting about among the plants. Melonworms are light green and have two fairly widely separated whitish lines that run the full length of the body. These white lines remain until the larvae are full grown, but disappear during the prepupal period. Melonworms confine their feeding largely to the foliage and flowers of cucurbits. When most of the foliage is destroyed, they may feed on outer portions of the fruit, but rarely tunnel out of sight. This insect is not as destructive as the pickleworm and is more easily controlled with insecticides.

Corn earworm.—Small corn earworms (*Heliothis zea* (Boddie)) are often found in the flowers of squash and sometimes in those of cucumbers. Corn earworms resemble pickleworms only when small, at which time their markings may look like the dark spots that are typical of small pickleworms. Larger corn earworms occasionally feed in squash flowers and on the surface of fruits of cucurbits.

Squash vine borer.—Small larvae of the squash vine borer (*Melittia cucurbitae* (Harr.)) are sometimes found in the flowers of squash and occasionally tunnel near the surface of the fruit. Most of the injury of this insect, however, is confined to the basal portion of the vines, where serious injury often is done. Squash vine borers are yellowish-white to almost white, grub-like caterpillars with dark brown to black heads. These larvae lack the spots that are typical of pickleworms and the white stripes that melonworms have.

PRACTICAL SIGNIFICANCE OF FINDINGS.—As pickleworms apparently survive the winter only in semitropical regions where host plants are available throughout the year, fall or winter cleanup measures would be of no value except possibly in winter breeding areas.

The gradual northward spread of the insect each spring suggests the possibility of annual predictions of infestations in a given area through careful surveys made in regions to the south, as well as through past experience.

The late appearance of the insect in many areas should make early planting of spring and summer crops a practical method of reducing its injury.

Movement of many larvae from the leaf and flower buds, where most of the eggs are laid, provides an opportunity to control the insect with an insecticide before it begins tunneling fruits and vines.

The short life cycle of the pickleworm during the summer and early-fall months, overlapping of broods, and tunneling habits of older larvae indicate that a relatively short interval between insecticide applications may be needed. As pickleworm moths do not lay eggs freely on plants that have not begun to bloom, applications can be delayed until the blossoms appear.

Summer squash is a favorite host, but not enough to make it of value as a trap crop in a cucumber or cantaloup planting.

Although a native creeping cucumber is a host of the pickleworm in the vicinity of Charleston, this plant does not appear to be sufficiently abundant or attractive to justify attempts to suppress it there or in similar geographical areas. Native host plants are probably a more

important factor in areas where they are abundant in midwinter.

No natural enemies that appeared to be of special importance were found.

SUMMARY.—The pickleworm (*Diaphania nitidalis* (Stoll)) is a destructive pest of cucurbit crops in several areas of the United States, especially in the Southeast. In conjunction with field-plot tests of insecticides, studies were made at Charleston, S. C., between 1948 and 1954 of those habits of the pickleworm most likely to have a bearing on its control. The various stages of the insect, its seasonal occurrence, and the nature of its injury are described.

All evidence obtained indicates that the species does not survive the winter in the Charleston area, but gradually spreads northward each year from semitropical areas, such as southernmost Florida, where host plants live at least through most of the winter. These findings agree with those of other recent workers in North Carolina and Georgia. The insect appeared in the Charleston area during May and early June in small numbers, gradually increasing in abundance until late fall. All cucurbits examined, including a native creeping cucumber, were found to be hosts, summer squash, cucumbers, and cantaloups being severely damaged during late summer and fall. Egg laying occurred chiefly after fruiting began. Practically no adults were found in cultivated fields during the day.

Efforts to obtain fertile eggs from reared adults were not successful. Field-collected eggs hatched within 3 days. The larval stage lasted 6 to 28 days, the pupal stage 6 to 70 days, and the entire life cycle, less the preoviposition period, 18 to 53 days.

The melonworm, corn earworm, and squash borer were often associated with the pickleworm and might be mistaken for it. Characters that distinguish these species from the pickleworm are given.

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SCIENTIFIC NOTES

Tests with Phosphorus Insecticides for Control of Pink Bollworm and Some Other Cotton Pests, 1955[1]

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Small field-plot experiments were conducted in Burleson County near College Station, Texas, in the Brazos River bottoms during the 1955 season to evaluate various phosphorus compounds primarily for the control of the pink bollworm, *Pectinophora gossypiella* (Saund.). This area afforded an excellent opportunity for conducting these tests since heavy infestations developed in many of the fields. Although these experiments were conducted primarily for control of the pink bollworm, the boll weevil, *Anthonomus grandis* (Boh.), and the bollworm, *Heliothis zea* (Boddie) occurred in sufficient numbers to evaluate the insecticides for control of these pests. Emphasis was given to Bayer 17147, since this material was highly effective against the pink bollworm in the laboratory and in preliminary field tests conducted at College Station (Ivy *et al.* 1954) during the latter part of the growing season of 1954.

Bayer 17147 alone and in combination with DDT, endrin, Bayer 16259, or DDVP plus Aroclor 5460 (a chlorinated terphenyl) plus endrin were used in these experiments. The DDT-dieldrin mixture was used as a standard of comparison.

All insecticides were applied in water emulsions with knapsack-type sprayers equipped with three nozzles per row. Approximately 6 gallons of the emulsion were applied per acre at 7-day intervals except when showers interfered. In experiment 1 there were six replicates of each treatment, three in each of two fields. In experiment 2 there were four replicates of each treatment in one field. All plots were 1/10 acre in size. All infestation records were made in the usual manner. The yield records were made from 0.04 acre in the center of each plot.

The infestation and cotton yields records for experiment 1 are shown in table 1. It will be noted that Bayer 17147 applied alone at the rate of 0.98 pound per acre and a mixture of 17147 and DDT were equally effective against the pink bollworm and gave the best control. Bayer 16259 at 0.50 pound, 17147 at 0.49 pound, and a mixture of dieldrin and DDT gave about the same degree of control. Endrin alone and the DDVP-Aroclor-endrin mixture gave very little control.

The injured-boll records indicated that 17147 at 0.98 pound per acre gave about the same control of the bollworm as the dieldrin-DDT mixture.

The phosphorus compounds (Bayer 17147, 16259, and DDVP) gave good control of a light infestation of aphids that developed near the close of the experiment.

Table 1.—Effect of insecticide sprays against the pink bollworm and the bollworm, Experiment 1.

Treatment	Pounds per Acre	Pink Bollworm		Bollworm	Pounds of Snapped Cotton per Acre	
		Per Cent Boll Infestation	Mines Per Boll	Per Cent Boll Injury	Yield	Gain
Check	—	76.0a[x]	8.63	5.1a	2046	—
Bayer 17147	0.49	36.5c	1.18	1.5b	2462	416
	0.98	22.0d	0.55	1.1b	2308	262
Bayer 17147+DDT	0.53+1.07	23.0d	0.56	1.1b	2662	616
Dieldrin+DDT	0.26+2.06	38.5c	1.33	1.7b	2862	816
Endrin	0.40	63.5b	5.01	1.7b	2471	425
Bayer 16259	0.50	37.0c	1.16	2.3b	2566	520
DDVP+Aroclor+endrin	0.50+2.00+0.25	66.0b	5.49	2.3b	2483	437

[x] Angle transformation used to determine L.S.D. (Snedecor 1946). Means followed by the same letter are not different at the 5% level of significance.

Table 2.—Results of insecticide sprays for control of the pink bollworm, boll weevil, and bollworm, Experiment 2.

Treatment	Pounds per Acre	Pink Bollworm		Boll Weevil	Bollworm	Pounds of Snapped Cotton per Acre	
		Per Cent Boll Infestation	Mines per Boll	Per Cent Squares Punctured	Per Cent Injury	Yield	Gain
Check	—	80a[x]	3.82	53a	8a	1519	—
Bayer 17147+DDT	0.26+1.00	33c	0.64	30b	3a	2181	662
Bayer 17147	0.48	34c	0.68	26b	6a	1675	156
	1.10	24c	0.46	21b	4a	2137	618
Dieldrin+DDT	0.26+2.10	32c	0.77	25b	4a	2387	868
Endrin	0.38	59b	2.32	27b	4a	1806	287

[x] Angle transformation used for determining L.S.D. (Snedecor 1946). Means followed by the same letter are not different at the 5% level of significance.

The dieldrin-DDT and 17147-DDT mixtures, and 16259 produced the highest yields, and 17147 applied at 0.98 pound per acre the lowest. No explanation can be given for the relatively low yield from the last treatment.

The infestation and cotton-yield records for experiment 2 are summarized in table 2. Bayer 17147 gave slightly better pink bollworm control at 1.1 pounds than 0.48 pound per acre, and better than either of the mixtures containing DDT. There was, however, no significant difference in control between any of these treatments. Endrin alone gave only a slight reduction in the pink bollworm infestation. All the insecticides gave good control of the boll weevil and were equally effective. The bollworm infestation records show that all the insecticides gave a

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[2] Assisted by Thomas E. Bonorden, Entomology Research Branch, in cooperation with the Texas Agricultural Experiment Station.

significant reduction in boll injury except the 17147 applied alone at the rate of 0.48 pound per acre.

Bayer 17147 gave good control of a light infestation of aphids and spider mites. Bayer 17147 at 1.1 pounds per acre and the dieldrin-DDT and 17147-DDT mixtures produced higher yields than the 17147 at 0.48 pound per acre or the endrin alone. This experiment was conducted in an irrigated field, and there was considerable variation in the growth and fruiting of the cotton due to unequal distribution of the water. The difference in yields between treatments, therefore, cannot be attributed entirely to insect control.

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A Partial Analysis of Cotton Extrafloral Nectar and Its Approximation as a Nutritional Medium for Adult Pink Bollworms[1]

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It has been known for some time that the adult pink bollworm, *Pectinophora gossypiella* (Saund.), like so many other insects, will feed on a simple sugar solution under laboratory conditions. This fact created the following questions: Assuming that the moth will feed in the field, just what carbohydrates are easily available in the average cotton field, and of what value is such a diet to the insect?

Among the possible sources of carbohydrate in cotton fields are secretions from floral nectaries, secretions from extrafloral nectaries, and honeydew from homopterous insects. Floral nectar and honeydew are well known for their nutritive qualities. However, neither of these exudates is present throughout the season in any consistent quantity and could only be depended upon during the blooming period or during heavy insect infestations. Further, the blooms open only during the day and, as the moth is nocturnal, the floral nectar is practically unavailable. Extrafloral nectaries are present throughout the season on either the floral bracts or the leaves, or both, but little is known about their copious secretions. It was decided, therefore, to analyze the extrafloral nectar, both from the floral bracts and from the vegetative growth, for carbohydrates, amino acids, and protein content. Further, a sugar solution simulating this nectar would be formulated to determine whether the moths would feed upon it.

Samples of nectar were collected by aspirating and pooling simultaneously many hundred droplets from Empire cotton plants in the field, and then stored in the laboratory until analyzed. The samples of the extrafloral nectar were very viscous, crystal clear, and in one sample taken from the floral area an amorphous white mass formed which consisted of approximately 25% of the total volume of the sample. At the time of collection there were no visible differences between the samples collected from the bracts and from the leaves.

Qualitative analyses were carried out by the use of the ascending paper chromatographic technique of Williams & Kirby (1948) using 6×7 inch Whatman No. 1 filter paper to increase their sensitivity (Clark & Ball 1952). The carbohydrates were analyzed by means of the one-dimensional technique, but the two-dimensional technique was used to separate the amino acids and proteins. The solvent used in the carbohydrate analyses was phenol (saturated with water) or butanol-acetic acid-water (4:1:5 v/v) (Patridge 1948). For the amino acid and protein analyses, phenol (saturated with water) and, following Bentley & Whitehead (1950), either propanol (70%) or acetone (60% with 1% w/v of urea) were used. The sugars were detected by the use of a beta-naphthylamine reagent (Novellie 1950). The amino acid and proteins spots were developed by spraying the paper with ninhydrin (0.2% w/v) and acetic acid (1%) in *n*-butanol (Consden *et al.* 1948). The chromatograms were dried at room temperature to avoid any loss of materials due to destruction by heat (Brush *et al.* 1951). The R_f values for the amino acids were no different from those values found in Clark & Ball (1952). No R_f values for sugars were determined, as identification was carried out by chromatograming knowns and unknowns simultaneously.

The chromatographic analyses showed the presence of the following sugars: Sucrose, glucose, fructose, ribose, rhamnose, and raffinose. The first three were present in appreciable amounts, and the others sporadically and only in traces. No amino acids were found even when large amounts of the unknown were placed on the paper.

A solution of these sugars was then prepared in the following proportions: Sucrose 40, glucose 30, fructose 20, raffinose 5 rhamnose 5, ribose 2, and water 100. These proportions were selected on the basis of the relative densities of the spots on the papergrams. This diet was placed in vials, which were stoppered with absorbent cotton and inserted into corks. These units were then inverted and pushed into holes in the tops or sides of the rearing boxes. Two hundred and ten moths were placed in the boxes, and observations were made to see if they would feed upon the diet and whether it affected their longevity, fertility, fecundity, or some other biological function. Water was used as a check.

To determine the number of moths that fed, the same procedure was followed with a diet of the same composition made radioactive with phosphorus^{32} (0.025μc/μl). After this diet had been available to 5000 moths for 3 days, they were examined for radioactivity. Further, a random sample was dissected to establish the position of the radioactivity in the internal organs.

The moths fed readily on the sugar diet, and it was noted that they began feeding the first night following emergence as adults. The sugar diet had no apparent affect on the longevity, but the egg output showed an increase of approximately 100% over the check (Lukefahr & Griffin 1956). In the experiments with the radioactive sugar diet no untagged moths were found. The tagged moths showed a wide distribution of activity, the midgut being most highly radioactive.

The results create some interesting speculation. As an increased fecundity is an asset to the furtherance of the species, it is reasonable to assume that these laboratory findings occur in the field. With this assumption, a nectarless cotton would be a great aid in the cultural control of the moth. Such a cotton variety might significantly reduce the seasonal rate of increase in the pink bollworm population, the reduction being dependent upon the decreased egg-laying potential. To what degree it could be reduced also depends upon other factors, including predation, parasitization, weather, population pressure, available food, and other environmental influences affecting the survival of the eggs and larvae. Thus, even if the decrease in fecundity under laboratory conditions is approximately 50%, it can only be assumed that the reduction in the field should be between 0 and 50%.

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The Effects of Food on the Longevity and Fecundity of Pink Bollworm Moths[1]

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In studying the life history of the pink bollworm, *Pectinophora gossypiella* (Saund.), it was necessary to supply the adults reared in the laboratory food similar to that occurring in nature. Since the pink bollworm is almost host-specific to cotton, the logical source of food was the nectaries of the cotton plant.

Trelease (1897) observed moths of the cotton leafworm, *Alabama argillica* (Hub.), and bollworm, *Heliothis zea* (Boddie), feeding readily on the nectar of the cotton plant and thought this to be an important source of food. Fletcher (1929) found the bollworm moth readily attracted to cotton nectar. He reported that plants growing on rich bottom-land soil had a greater flow of nectar and a higher bollworm infestation than plants growing in less fertile soils. Nuttycombe (1930) found plant nectar to be an important source of food for the corn earworm moth. Quaintance & Brues (1905) reported that the bollworm moth does not begin to oviposit until the female has been able to partake of food.

Since it had been established that the bollworm and leafworm moths feed on cotton nectar, it was assumed that the pink bollworm moths would do likewise. To determine what effect the food from the cotton nectaries might have on the longevity and fecundity of the pink bollworm moth, a series of tests were designed to compare simulated cotton nectar with other diets.

MATERIALS AND METHODS.—The chambers used for confining the moths during these studies consisted of large Dietz Pioneer lantern globes resting on moist sand in a porous clay pot. For each globe a half-pint small-neck bottle containing plant-nutrient solution was embedded in the sand. The sand was covered with a piece of black sateen cloth with a small hole in the center so that a cotton-plant terminal could be inserted into the bottle. The globe rested on this cloth, and the top was covered with small-mesh cheesecloth. A small glass jar containing food, with a cotton wick protruding slightly from the top, and a pair of newly emerged moths were placed in each globe.

The globes were set up and divided into four groups with regard to the food supplied. The moths in the first group had access to a solution containing the six sugars found in the extrafloral nectaries of Empire cotton (Clark & Lukefahr 1956), those in the second group to a 50–50 mixture (by weight) of honey and molasses, in the third group to a similar mixture of honey and molasses plus 5% of protein hydrolyzate (by weight), and in the fourth group to water only. The globes were checked daily for moth mortality, and every third day the plants were removed, checked for eggs, and replaced with new plants. When the females died, they were dissected to determine the number of mature eggs remaining in their ovaries.

An analysis of variance was performed on the data. The analyses on the number of eggs laid and the number of mature eggs in the ovaries of the dead females were performed on a transformed variable.

RESULTS.—The data obtained on the longevity and fecundity of the moths are summarized in table 1. Those feeding on the various combinations of sugars laid more eggs than those feeding on water only, the increase ranging from 37 to 54%. The number of eggs deposited by the females feeding on the simulated cotton nectar was significantly greater than the number laid by those on the water diet. The honey-molasses and honey-molasses plus protein hydrolyzate diets gave intermediate numbers of eggs between these. The difference in either direction were not significant; however, the numbers for these two diets were closer to that for the cotton nectar. There was no significant difference in longevity between the males or females regardless of regimen. Furthermore, the diets had no significant effects on the number of eggs remaining in the dead females or the percentage of fe-

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Table 1.—Effects of various foods on the longevity and fecundity of pink bollworm moths.

Food	Males		Females			Mean No. Eggs		
	Number Observed	Average Longevity (Days)	Number Observed	Average Longevity (Days)	Number Copulating and Ovipositing	In Dead Females	Laid per Female	Of Eggs Laid (Transformed)[a]
Simulated cotton nectar	29	10.7	32	10.2	14	17	146	11.5
Honey and molasses	27	9.4	21	11.7	13	12	117	9.9
Honey, molasses, and protein hydrolyzate	26	9.2	26	10.5	13	18	107	9.8
Water	26	9.7	23	9.5	26	15	68	7.6
L.S.D. at 5% level	—	—[b]	—	—[b]	—[b]	—[b]	—	2.8
1% level	—	—	—	—	—	—	—	3.7

[a] Statistical analysis of the data on number of eggs laid and number of eggs in dead females was performed on a transformed variable. If x is the number of eggs, then $y = \sqrt{x+1}$ is the transformed variable. The transformation was performed on the components of the "Average Number Laid per Female," and not on the average of the components. The LSD's apply to the transformed variable.

[b] Not calculated since F test was not significant.

males mating and ovipositing. Protein hydrolzyate, as a source of protein in the moths' diet, did not increase longevity or egg production.

CONCLUSIONS.—These results indicate that the nectaries of cotton may be an important source of food for the moths in nature. Further work is warranted, including field tests with nectarless cotton such as the experimental variety developed at the North Carolina State College of Agriculture.

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Observations on the European Corn Borer and the Corn Earworm Infesting Early-Season Plantings of Country Gentleman Sweet Corn[1]

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Insecticidal control of the European corn borer, *Pyrausta nubilalis* (Hbn.), and the corn earworm, *Heliothis zea* (Boddie), attacking Country Gentleman sweet corn has been notoriously poor in Illinois. Four, five, and even six treatments of the DDT and oil spray solution suggested by Blanchard *et al.* (1951) and Gannon *et al.* (1955) resulted in only 10 to 40% of the ears free of damage from corn borer and corn earworm. Standard practices followed in planting this variety appeared to contribute greatly to the poor control obtained with insecticides.

Prior to 1954 very little Country Gentleman corn was planted in Illinois before May 20 because the principal variety, Illinois 13, was known to germinate poorly in cool or cold soils. May 20 was a rule-of-thumb date on which canners incorporated this variety into their planting schedule. This resulted in the corn silking and maturing late in the canning season during the period of greatest egg deposition by the corn borer and the earworm. During 1954, two canning companies co-operated in planting Country Gentleman sweet corn on sandy soils in their area well in advance of the May 20th date. In general, these plantings of corn were too young for the first-generation European corn borer and too mature for the second-generation corn borer and the corn earworm. Results with insecticides were good because heavy infestations of either pest did not occur in the early-planted fields.

In 1955, three canning companies followed suggestions to plant Country Gentleman early in the season. These canners advanced Country Gentleman planting schedules by 20, 12, and 10 days, beginning planting of this variety on April 30, May 8, and May 10, respectively. Country Gentleman corn planted after May 5 in 1955 received no treatment for first-generation corn borers and much of the acreage was treated only once or twice for second-generation corn borers and earworms. Country Gentleman corn planted on April 30 received only 13.4% of the total eggs laid by first-generation corn borers in the area under study. A comparison of a 75-day yellow variety, an 80-day yellow variety, and 90-day Country Gentleman planted on the same date and receiving no protection from first-generation corn borers showed that the Country Gentleman had 87.4% less trimmable ears than the 75-day variety and 60% fewer trimmable ears than the 80-day variety. During 1955 Country Gentleman corn harvested at the three co-operating canning companies averaged 90.5% of the ears free of damage from corn borer and corn earworm.

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A New Host of the Boll Weevil[1]

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The boll weevil, *Anthonomus grandis* Boh., is commonly thought to be host specific to species of plants belonging to the genus *Gossypium*. However, Coad (1913) observed a boll weevil feeding in a flower of *Hibiscus syriacus* L. and was able to rear several in the laboratory on this plant. Under laboratory conditions he also observed boll weevils feeding on *Callirrhoe involucrata* (Nutt.), *C. pedata* Gray, and *Sphaeralcea lindheimeri* Gray. Gaines (1933) caged *H. syriacus* in the field and was able to rear several weevils from it. Gaines (1934) found that the boll weevil would infest this plant naturally when it was interplanted with cotton in the field. Wormser (1944) reports the feeding of boll weevils on young okra pods, *Hibiscus esculenteus* L.

In July 1956 at Brownsville, Texas, the author noticed that several flower buds of *Thespesia populnea* (L.) Soland were turning yellow and falling off the tree. Upon examination he found that they were infested with what appeared to be boll weevil larvae. He also found four adult boll weevils on the foliage. All the flower buds and seed pods eventually became infested. Several of the infested buds were collected and 14 adult boll weevils emerged from them. The identification was verified by Rose E. Warner, of the Entomology Research Branch. All the adults were normal in size, which indicated an adequate food supply. Nine of them were placed in a cage containing potted cotton plants, and in a short time all the fruiting forms showed evidence of feeding or egg punctures.

This new host plant was being grown in the yard of a home fully three-quarters of a mile from the nearest cotton field. At the time it was found infested there was an abundance of cotton available in the fields, which indicates that this infestation was the result of a natural selection.

Thespesia populnea belongs to the family Malvaceae and is commonly called portia tree or locally is referred to as a tulip tree. It is a woody perennial grown for shade and ornamental purposes. According to Bailey (1941), it is a native of tropical Africa and Asia and in its native habitat reaches a height of 30 to 50 feet, blooming the entire year. The flower buds are as large as the squares of a cotton plant.

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Granulated Endrin for White-Pine Weevil Control During Hibernation[1]

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Tests for insecticidal control of the white-pine weevil, *Pissodes strobi* (Peck), generally have been directed against the adults during periods of their activity in the spring and early fall. Best results thus far have been obtained by early spring applications.

In the fall of 1955 the author made a preliminary test of a granulated form of endrin applied to the surface of the duff in a white pine plantation. Three treated plots and one check plot were laid out to include 10 cages per plot. These cages were 5-quart cans with the tops and bottoms removed. Each cage was pressed down through the duff layer into the mineral soil to a depth of 1 to 2 inches.

Each cage in the treated plots received a proportionate quantity of the insecticide to equal the amount desired per acre. Three dosages—1, 2, and 4 pounds of actual endrin per acre—were used. The insecticide was spread over the surface of the duff, within the confines of the cage, as evenly as possible on October 10.

Test weevils were placed in each cage immediately after treatment: 20 weevils per cage in plot 1 and 10 weevils per cage in the other three plots. Freshly cut pine twigs were placed in each cage to provide food for the weevils until they moved into the duff to hibernate. The tops of the cages were covered with plastic screening to prevent escape and the chance of wild weevils entering the cages.

The cages were left undisturbed, except to replace food material as required, until April 2, 1956. At this date the cages were carefully lifted, including all the duff material within them, and were brought into the laboratory. Fresh pine twigs were placed in each can and the weevils were allowed to emerge from the duff and feed. Examination of the cages was made daily and the emerged weevils were removed. Emergence from all cages was completed on April 11 and the results are given in table 1.

Under the conditions of the test, lateral movement outside the treated area was prevented by the cages. However, as the test was started relatively late in the fall, weevil movement was probably at a minimum due to the beginning of actual hibernation very soon after caging. This technique does not represent, necessarily, the reaction of the weevil to the insecticide under normal conditions. However, it does indicate that endrin used in this manner, showed considerable promise. Further testing is necessary to more fully evaluate this method of treatment.

Table 1.—Total emergence of test weevils from cages after hibernation, April 2 to April 11, 1956.

Plot No.	Pounds of Endrin Used per Acre	Weevils Used per Plot	Weevils Emerged per Plot	
			No.	Per Cent
1	1	200	2	1
2[a]	—	100	94	94
3	2	100	0	0
4	4	100	1	1

[a] Untreated check plot.

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Notes on the Biology of the Pea Aphid[1]

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Ever since Blanchard & Dudley (1934) and Painter & Grandfield (1935) observed that certain strains of alfalfa growing in fields were resistant to attacks of the pea aphid, *Macrosiphum pisi* (Harris), workers in various parts of the country have been attempting to breed and select resistant strains which can be grown commercially. In 1944 the Department of Plant Breeding at Cornell University initiated a program for developing new varieties of alfalfa, and in conjunction with this work, the Department of Entomology began tests for resistance to the pea aphid on these new strains.

The first series of tests were conducted in the greenhouse during March and April 1948 and consisted simply of transferring a number of aphids (the form of these were unknown) which had been reared on broad bean (*Vicia faba*) to replicated plots of alfalfa cuttings and taking counts of the progeny at the end of a week. The work was discontinued in April 1948 but was resumed in the spring of 1949. At this time it was noted that the aphids did not survive or reproduce on alfalfa cuttings. Similar failure to rear the aphids on alfalfa occurred during the fall and winter of 1949–50 and during the spring of 1951.

During the winters of 1952 and 1953 attempts to transfer the aphids from the bean plants to alfalfa plants were made again, but with no success. Several methods of transfer were used and the alfalfa plants were maintained under almost optimum conditions with regard to temperature, plant nutrition, pH of the soil and light intensity; however, all transfers ended in failure since the aphids either died on the alfalfa plants or migrated from them. When several pots of alfalfa were exposed to a light intensity of 10,000 foot-candles some of the transferred aphids remained on these cuttings and reproduced, though at a very slow rate (21 offspring from seven females in 17 days).

The pea aphid, unlike the typical migratory aphid which migrates to and from unrelated plants, is generally considered to be a nonmigratory species which lives on plants of the family Leguminosae such as *Vicia*, *Medicago*, *Trifolium*, and *Pisum*. In the numerous studies of this aphid reported in the literature no designation is made of the form of aphid used, whereas when migratory aphids are studied such forms as *fundatrigenae*, *aliencolae*, *sexuparae* are recognized. Mordwilko, however, (1928) does conclude that the pea aphid has a "facultative" migration from and to its primary and secondary host plants.

On April 15, 1954, several *fundatrices* were brought in from alfalfa in the field and placed on the alfalfa plants in the greenhouse to find out whether they would react differently from the aphids transferred from bean plants. Reproduction soon occurred and increased rapidly after the *fundatrigenae* were born. The fact that the spring forms of the pea aphid survived under these conditions, while the aphids taken from bean would not, indicated that the fall forms of the pea aphid (*sexuparae* and *sexuales*) should also survive on the alfalfa (primary host) in the greenhouse. Subsequently, *sexuparae* brought into the greenhouse in November 1954 and placed on the alfalfa plants settled and reproduced at a very rapid rate under suboptimum conditions of

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light, on overly mature plants, and with fluctuating temperatures and heavy parasitization.

This difference in behavior between aphids normally found on the primary host (alfalfa and clover) and aphids which feed on annual legumes in the summer seemed to show that the pea aphid is a migratory aphid after all. Both Patch (1927) and Davidson (1927) concluded that the pea aphid is a true migratory aphid, though this fact usually is not taken into account by workers using the pea aphid in their various studies. Patch states: "There seems to be no real inconsistency in applying the term migratory to the pea aphid in the north where it annually migrates between a primary and secondary host, and the pea aphid may be considered an exception in that its primary and secondary host plants belong to the same plant family." Although most biological workers do not call *pisi* a "true migratory aphid" they do state that it lives on different legumes in the summer than during the rest of the year.

It would not be too unreasonable to assume that each form of the aphid has a decided preference for a type of host plant. Hence the *fundatrix* hatching out of the egg in the spring on alfalfa selects that plant, while later as the alfalfa plant slows in growth and becomes mature a selection for more succulent hosts occurs and winged migrants develop which fly to and settle on annual legumes. Eventually, as these summer legumes mature and dry out, fall migrants develop and fly back to alfalfa (or other perennial legumes) and produce the sexual forms which deposit the overwintering eggs.

From the assumption that the aphids maintained on *Vicia* in the insectary were the summer forms (*aliencolae*), though possibly they could have been a biological race (Harrington 1945) an attempt was made to get them to produce *sexuparous* form during January 1955. Several pots of young bean plants infested with *aliencolae* were placed in a room kept at a temperature range of 35 to 50 degrees F. The only light available to the plants came through a window on the northeast side of the room. Under these conditions the bean plants did not grow appreciably, though the aphids fed and reproduced. At the end of 5 weeks the aphids were transferred to alfalfa plants where they settled immediately and reproduced at a greater rate than on the dormant bean plants, indicating that *sexuparae* had been produced.

The *fundatrices*, *fundatrigenae*, *sexuparae* and *sexuales* of the pea aphid feed and reproduce on such primary hosts as *Medicago*, *Trifolium* and *Lotus*, and can probably survive on the summer annuals in the greenhouse. The winged migrants which fly from the primary host (alfalfa and clover) to the secondary host in the spring and back to the primary host in the fall are probably intermediate in their host plant preferences, but the summer form is more discriminate and prefers only the succulent growth of the annual legumes. Small populations of the pea aphid can be found on alfalfa during the summer in the field in New York and this is possible because, under the conditions of summer, alfalfa approaches peas and beans in so far as nutritional requirements of the aphid are concerned. Hence, several *aliencolae* were able to settle and reproduce on alfalfa plants subjected to light intensity of 10,000 foot-candles. Nevertheless, alfalfa plants under greenhouse conditions in the region of Ithaca, New York, would be very different from alfalfa growing in the field and would not be satisfactory for *aliencolae* growth and survival.

A knowledge of the form of pea aphid used in such studies as virus transmission, nutrition, ecology and, particularly, plant resistance would be of great benefit to the outcome of these studies.

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Resistance of the Southern Potato Wireworm to Insecticides[1]

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Studies of wireworms as pests of vegetables in the south Atlantic coastal plains were begun at Charleston, S. C., in 1952. As the major problem appeared to be their injury to Irish potatoes, most of the work has involved that crop. According to Deen & Cuthbert (1955), the southern potato wireworm, *Conoderus falli* Lane (formerly *C. vagus* Cand.), is the most abundant species in most commercial potato-producing areas of the Southeast. Evidence of resistance to certain insecticides in this species in the Charleston area is reported herein.

FIELD STUDIES.—Field-plot evaluations in 1952 showed that aldrin, dieldrin, and heptachlor at 2 pounds and chlordane at 4 pounds per acre provided effective control. These results were substantiated by similar tests in 1953 and 1954. Commercial usage of chlordane for wireworm control in potato plantings began in 1952 and became almost universal by 1953. The dosages ranged from 1½ to 4 pounds per acre, and in practically all fields results were entirely satisfactory through 1954.

First evidence of a decline in the toxicity of chlordane was noted in 1955. The certificates of Federal-State market inspectors showed that, of about 975 lots of potatoes examined during the harvest of the spring crop, 14% were ineligible to U. S. grade 1 chiefly because of wireworm damage (as defined in U.S.D.A. market standards) and that wireworm damage constituted 50% of the defects that caused an additional 4% of the lots to be out of grade. The current tolerance for all defects allowed for that grade is 6%. Most of the fields had been treated with chlordane.

The experimental plots of 1955 did not provide data that can be compared directly with those of the three preceding years, but it was evident that the applications of aldrin, chlordane, dieldrin, and heptachlor made that year were not as effective as before and that there was a marked decrease in the effectiveness of residues from earlier applications.

The decline in the effectiveness of these four materials was even more pronounced in 1956. Observations on the commercial usage of chlordane up to 5 pounds per acre and of dieldrin up to 2½ pounds indicated that adequate control was obtained only where wireworm populations were low. Dosages that had previously been effective proved inadequate against moderate to heavy infestations in experimental plots. In spite of the use of chlordane, aldrin, or dieldrin on practically all commercial plantings in the Charleston area, the inspection certificates showed that, of 1,035 lots examined, 30% had sufficient wireworm damage to be ineligible to U. S. grade 1 and an additional 36% had 3 to 6% wireworm damage.

The degree of protection to potato tubers against wireworm injury provided by these insecticides in experimental plots during 1952, 1953, 1954, and 1956 is shown in figure 1. The insecticides were broadcast over the surface and mixed into the soil with a disk or rotary harrow shortly before the crop was planted in January or February.

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[2] In cooperation with the South Carolina Agricultural Experiment Station.

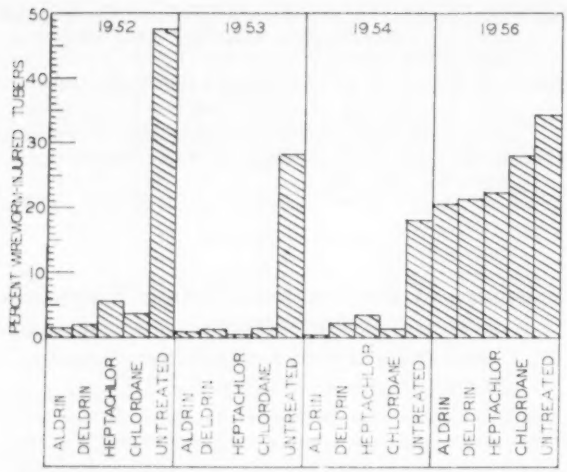

FIG. 1.—Per cent, by weight, of potato tubers injured by wireworms following soil applications of certain insecticides. Chlordane was used at 4 pounds per acre, dieldrin and heptachlor at 2 pounds, and aldrin at 1 pound in 1953 and 2 pounds the other years. Previously untreated field plots were used in 1952, 1953, and 1954. The 1956 plots received identical applications the preceding year.

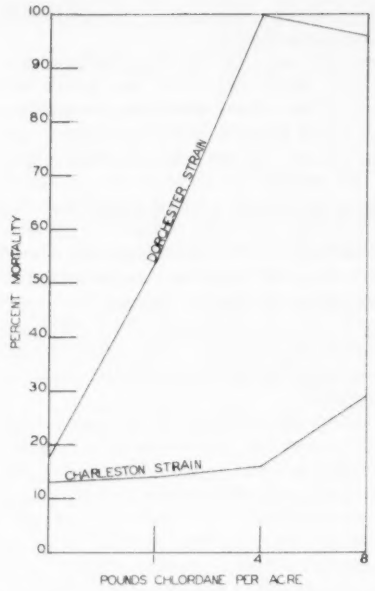

FIG. 2.—Relative susceptibility to chlordane of two strains of the southern potato wireworm in 1956 cage tests.

LABORATORY STUDIES.—Comparisons of the susceptibility to chlordane of field-collected southern potato wireworms from Charleston County with that of specimens from adjoining Dorchester County were begun in March 1956. Adults of both groups were identified by M. C. Lane of the Entomology Research Branch, who noted no external differences between them. The Charleston larvae came from potato fields that had received several annual applications of chlordane; those from Dorchester County came from fields near Summerville and an area where potatoes are not grown commercially and where little or no chlordane or other soil insecticides has ever been used. The two areas are approximately 25 miles apart, and much of the intervening land is heavily wooded.

Individual larvae were caged in 3- or 4-ounce salve cans containing soil treated with chlordane at dosages equivalent to 0, 1, 4, and 8 pounds per acre, 6 inches deep. From 22 to 27 larvae from each area were exposed to each dosage. The cages were examined weekly, and food and moisture were added. The test was terminated on August 3, by which time all the survivors had emerged as adults. The striking difference in the susceptibility to chlordane of the wireworms from the two areas is shown in figure 2. Almost complete mortality of the Dorchester strain was caused by the 4- and 8-pound dosages, whereas the 4-pound dosage resulted in little or no mortality of the Charleston strain, and the 8-pound dosage killed less than 30%.

DISCUSSION.—It appears that the southern potato wireworm population in potato-producing soils in Charleston County has developed a rather high resistance to chlordane following several annual soil applications of that insecticide. Its use in the area began about 1948 and became general by 1953. It also seems apparent that this wireworm has developed at least moderate resistance to aldrin, dieldrin, and heptachlor, even though there were practically no commercial soil applications of these materials in the area prior to 1956.

Limited studies indicate that not more than two generations of this wireworm occur annually in the Charleston area. The development within such a few years of resistance to an insecticide in an insect with a relatively long life cycle is unusual. It probably was due partly to the fact that the chlordane applications made prior to 1952 for the control of mole crickets probably were sublethal to many, if not most, of the southern potato wireworms. No other published report of apparent resistance by a wireworm to a soil insecticide has been noted by the writers. However, a recent letter from Dale M. Norris, Jr., entomologist at the University of Florida Potato Investigations Laboratory, indicated that insecticide resistance may be developing in the southern potato wireworm in the vicinity of Hastings.

No evidence of decreased effectiveness of DDT against this wireworm has been noted. Limited field tests in 1952 and 1953 indicated that DDT at 10 pounds per acre was inferior to chlordane at 4 pounds. DDT at 20 pounds was not as effective as dieldrin at 1 pound the first season applied (1953), but provided equally adequate control in 1954 and 1955, and was superior in 1956. In other limited field tests DDT at 20 pounds proved superior to chlordane and dieldrin at 2 pounds and to heptachlor at 3 pounds per acre in 1956.

The Gulf wireworm, *Conoderus amplicollis* (Gyll.), has shown no tendency to develop resistance to any of the insecticides discussed in this article. This species constituted 3% of the adults reared from larvae collected from potato-producing soils in Charleston County between 1952 and 1954, and appears to be second in economic importance among the wireworm pests of potatoes in that area.

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EDITORIAL

New Trends in Plant Quarantine

When Professor E. O. Essig wrote the interesting treatise on plant quarantine that appeared in Science early in 1930, he stated that it was prepared "with the aim of presenting the claims of this young member of the administrative family." Now, a generation later, the claims of this maturing member of the regulatory family are being constantly reappraised and adapted to the changing American plant pest scene.

Several recent trends can be attributed to the airplane, which is at the same time the villain and a benefactor. It has undoubtedly helped bring in at least a few of our recently arrived noxious pests. It has also been of major assistance in quarantine-control campaigns to wipe out unwelcome insect migrants. The potentiality for plant pest introduction by plane is illustrated by interceptions made in a plane from Paris arriving on June 26, 1956. Plant quarantine inspectors who searched the passenger, baggage, and cargo compartments upon arrival at New York collected 45 live adults of the European chafer. Crew members stated that during their stopover in Paris chafers were swarming around the lights at the airport. On the constructive side the airplane has made feasible a long term plan for eradication of the gypsy moth in the New England States and is a major factor in the current Mediterranean fruit fly eradication program.

Definite, progressive advances in plant quarantine practices are well illustrated by comparing the eradication campaign against the Mediterranean fruit fly in Florida in 1929 with the current activity. Although the objective—complete eradication—is the same, plant quarantine philosophies and methods are vastly changed. The 1929 campaign involved an almost frenzied "scorched-earth" policy to rid the area of all host material, cultivated or wild, ornamental or commercial. Considerable public resentment was engendered because of the compulsory destruction of crops without compensation and drastic interference with commercial fruit and vegetable production. The urge for quick eradication is just as imperative now, but the methods of attack are radically different. The contention now is that the fly should have easy access to hosts to discourage distant flight. Quarantine regulations now recognize that established spray schedules applied to vegetable crops are so effective that they eliminate all sprayed vegetables in sparsely infested areas as potential sources of spread. Soon after an infestation is discovered by means of the greatly superior lures now used, an airplane swoops in with a lethal poison-bait spray that even with incomplete coverage will attract and kill nearly 100% of the adult flies in the general vicinity. Repeated at frequent intervals, there is a sudden, near-eradication of fly population.

There is also a tendency for research to come forward with fresh approaches to plant quarantine problems. For example, a 3-year cooperative research project on the effectiveness of modern cotton gins in killing pink boll worms has demonstrated that most of the worms are killed in the ginning process. This has permitted the substitution in Texas and New Mexico of more economical treatments for the previous requirement that cottonseed be heat-treated as a part of the continuous process of ginning. This has saved the growers and industry $1\frac{1}{2}$-million annually and has permitted reassigment of personnel.

Another impetus is the increasing pressure from State plant quarantine officials and from industry for Federal participation in most plant pest problems, even those confined to a single State. Recently this has been apparent in the unanimous recommendations for Federal quarantine measures at public hearings held to review the khapra beetle, European chafer, and Mediterranean fruit fly situations. The greatest vote of public confidence ever manifest in behalf of a Federal quarantine was the response in 1951 to a formal proposal that the Japanese beetle quarantine be revoked and the activity remanded to infested States. Congressional and other reactions were positive and decisive.

Undertakings and accomplishments of the khapra beetle quarantine-control project indicate some of the audacity of modern quarantine methods. The immensity of the task of wiping out this grain-destroyer might well have been regarded as impossible of accomplishment in the 1930's. Means of covering a million cubic feet of quarantined warehouse and of dispensing tons of methyl bromide fumigant were then virtually unknown.

Still another trend is the increasing intricacy of promulgating plant quarantines. Thirty years ago the chairman of the Federal Horticultural Board or any one of several staff members could sit down and with a reasonable degree of fluency write a domestic or foreign plant quarantine. After the legally required public hearing, the quarantine could be made immediately effective. The criteria then were that the order was reasonably biologically sound and legally sufficient. Nowadays the procedure is both tortuous and diffuse. For example, the Federal Register Act required the codification in 1939 of all administrative orders and regulations. Legal terminology has been progressively introduced in regulatory orders. Adding to the routine is the Administrative Procedure Act of 1946, which requires an advance notice and comment period for all administrative actions and a deferred effective date when finally they are adopted. The reorganization of the Department of Agriculture in January 1954 eased the situation somewhat. Now quarantines and regulations formerly requiring the signature of the Secretary of Agriculture may be approved by the Administrator or Deputy Administrator of the Agricultural Research Service. In some instances there is a further delegation of authority to the Chief of the Plant Quarantine Branch or the Plant Pest Control Branch in order to relieve certain restrictions or to establish regulated areas within the bounds of an already quarantined State.

Adding to plant quarantine complexities is a tremendous increase in the means for further introductions of the golden nematode into the United States. Soil-contaminated salvaged war material, and returned auto-

mobiles that have been driven in nematode-infested foreign countries and have dirt adhering to the undercarriage, are troublesome current problems. Excellent cooperation has been obtained from the military services and steamship lines in voluntary precleaning of equipment and vehicles at the ports of shipment prior to loading.

Virus-disease problems have established still another tendency—in this instance, a pronounced tightening in recent years of several import restrictions. For example, there is now a flat prohibition on the importation of *Prunus* spp. from Europe, Asia, Africa, and Oceania, to prevent the entry of a diversity of plant diseases. Among this diversity are a number of viruses. The public has become acquainted with this particular embargo because of the Department's persistent refusal to authorize importation of scores of gift shipments of Japanese cherry trees destined to America cities. Thus, on a sound biological basis, high-risk material is being effectively excluded and our plant quarantine protection strengthened.

A three-year lapse in complete Customs baggage inspection has stimulated one phase of a movement toward strengthening plant quarantine port inspection. In April 1953, because of increased workloads beyond the capacity of their available manpower, the Bureau of Customs substituted spot checking for a complete inspection of all arriving passengers' baggage. Interceptions of contraband plant material dropped off immediately. During this 3-year period the Mediterranean fruit fly gained entry into Florida. Since one of the heaviest infestations straddles the Miami International Airport, some authorities are of the opinion that lack of adequate baggage inspection permitted the fly to gain entry there. This situation has been remedied. The 1957 fiscal year agricultural appropriation act contained an item of $800,000 that was transferred to the Customs Service to enable that agency to restore complete baggage inspection. When this was done there was an immediate, tremendous increase in the number of plant contraband interceptions from passengers' baggage. The 1957 appropriation also contained an increase of over $100,000 to augment the port-of-entry inspection force.

An improvement that may materialize next year is the modernization of the Insect Pest Act. The 1905 act forbids the transportation interstate, or from a foreign country, of notoriously injurious insects, as such. A bill was introduced in the House of Representatives during the 84th session that would legalize restrictions on the entry of dangerous organisms of any kind. This would permit restrictions on the entry of soil and other nonnursery items not now covered by the Plant Quarantine Act of 1912.

There is also a greater emphasis on remote-control, preventive plant quarantine measures. For example, what may be regarded as a south-of-the-border holding action is the neighborly cooperative work that the United States and the Mexican Departments of Agriculture are doing to prevent the northward migration of the citrus blackfly and the Mexican fruit fly and the westward extension of the pink bollworm into Baja California. Then there is the off-shore fruit fly research work performed in Honolulu that has furnished bases for the newly improved techniques now being successfully employed in fighting the Mediterranean fruit fly in Florida. Central American countries have also been most cooperative in inviting a survey by U. S. plant quarantine entomologists of their fruit-growing areas. Inaugurated to determine possible spread of the Mediterranean fruit fly from Costa Rica, the survey, in which entomologists of the surveyed countries cooperated, showed a complete absence of the fly in all other Central American countries. Similar cooperative surveys were also conducted in Cuba, the Bahamas, Jamaica, and the Dominican Republic.

Another modern concept in international plant quarantine cooperation is the preshipment inspection abroad by American inspectors of Dutch, Belgian, and French-grown bulbs. This greatly increases the effectiveness of the inspection with no increase in inspection costs. In this activity the plant quarantine services of both exporting and importing countries are cooperating to the mutual advantage of each. This activity is also illustrative of the close liaison between the Federal service and the National Plant Board. The chairman and vice chairman of the Board were asked to make an on-the-spot survey of these activities. This they did in August 1956.

Preflight quarantine inspection of planes departing from Hawaii and Puerto Rico for the mainland is another off-shore bulwark against our insect enemies. Through an agreement with the respective countries, our inspectors give similar preflight clearance to planes en route from Hawaii to Australia and New Zealand.

The late E. Ralph Sasscer was largely instrumental in transforming the function of the Federal plant quarantine inspector from a police-like role to that of a highly trained professional specialist. This progressive move has been furthered by the establishment at the Port of New York of a Plant Quarantine Branch Training Center. This center gives intensive training in technical and regulatory aspects of the work, enabling new appointees to take over journeyman inspectors' duties in much less time than was required during the former on-the-job training. Also, the skills and knowledge acquired enable them to function more efficiently. A further stimulus to this field of activity are the plant quarantine courses offered at several universities and colleges, including the University of Minnesota, University of Florida, and Colorado A. & M. College.

A further step in international cooperation that will eventually accrue to the benefit of the United States is the training of foreign nationals in plant quarantine procedures and techniques. Sponsored by the Foreign Agricultural Service, this is conducted at the Plant Quarantine Branch's Training Center at New York. Some 30 trainees from 15 different countries have completed this course. Upon return to their homelands, these trainees will establish plant quarantine services there if none exist or will strengthen existing services. By thus preventing the international spread of plant pests, they will indirectly aid this country's plant protection activities.

More effective education of tourists in the responsibilities for protecting this country from foreign pests is another field now being given increasing emphasis. A reference to plant quarantine requirements is now contained in a new, simplified Customs baggage-declaration form. Information on the subject is also contained in a leaflet

"Custom Hints" that is widely distributed to American tourists. A color, sound motion picture designed to inform the traveling public of the need for plant quarantine enforcement will soon be available to TV stations, transportation agencies and other interested groups.

These diverse trends are integral parts of greatly improved plant quarantine procedures. As such, they indicate realistic reappraisals of the entire field, with the adaptation of many innovations to protect the United States against the introduction and spread of foreign plant pests.

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BOOK REVIEWS

CHEMISTRY AND USES OF PESTICIDES. By E. R. deONG. 2nd Edition. vii+334 pages. Reinhold Publishing Co., New York. 1956. $8.75.

The first edition of this book was entitled "Chemistry and Uses of Insecticides." The author has included fungicides, herbicides, rodenticides, repellents and seed disinfectants in the present edition. To make room for this material the chapters on "Arsenical Compounds," "Copper Compounds," "Sulfur and Its Compounds," "Miscellaneous Inorganic Compounds" and "Petroleum and Its Products" have been rewritten and condensed. A new chapter "Organic Herbicides and Rodenticides" has been added and the other pesticides are included in the chapter "Synthetic Organic Insecticides and Fungicides; Repellents; Seed Protectants." Almost half of the book is devoted to these two chapters. The chapters, "Fumigants and Fumigation," "Plant Derivatives," and "Cold, Heat, Dehydration and Radiation as Pesticides" have been rewritten and expanded.

The style of the book remains the same as in the first edition. The pesticidal chemical is given and this is followed by its physical properties, formulations and uses. The ample index is used to direct the reader to the principal chemicals used in the control of any given pest. This system is not followed in the case of repellents, to which subject only two and a half pages are devoted. The allied subject, attractants, is neglected entirely.

The Appendix contains a useful "Dictionary of Pesticides," a summary of the Food and Drug tolerances of pesticide residues on fresh fruits and vegetables, and antidotes to be used in case of accidental poisoning with pesticides.

This handbook should be very useful to anyone concerned with production or use of pesticides. The information on each individual pesticide is well organized and easy to locate. Each chapter is backed up with well chosen references which direct the reader to more detailed information.

ELMER E. FLECK

SOIL ZOOLOGY, D. K. M. Kevan, Editor. 1955. Butterworths Publications, Ltd., 88 Kingsway, London, and Academic Press, Inc., 125 East 23rd St., New York 10. XIV+512 pages, 171 figures, 34 tables. $10.00.

The sub-title of this book is: "Proceedings of the University of Nottingham Second Easter School in Agricultural Science, 1955." It is a compilation in modified form of the 24 papers on various aspects of the broad but vaguely defined field of "soil zoology" which were presented at the Second Easter School in Agricultural Science held on April 1 to 7 inclusive, 1955. Also several contributions which were read only by title there are included, plus concise descriptions of a number of demonstrations which were on display at the meeting. Fifty chapters are included, although a number of them are brief (four with two pages, and five each with three and four pages, respectively). Most chapters list pertinent references, and there are comprehensive author and subject indexes at the end. The oral discussions which followed the formal presentations are summarized at the ends of the appropriate chapters, and many chapters begin with short italicized summaries. A unique and valuable feature is a 37-page illustrated key in the form of an appendix which covers the gamut of soil animals from vertebrates to protozoa.

What is soil zoology? A few statistics on the contents of this book indicate the present state of our knowledge of soil animals and where most of the research emphasis has been directed. Exclusive of general discussions, the following groups are covered by the approximate number of pages indicated for each: nematodes, 66; earthworms, 42; Collembola, 29; Enchytraeid worms, 20; mites, 18; protozoa, 15; millipedes and centipedes, 14; microflora, 13; termites, 11; and Symphylids, 7. A further break-down on the numbers of pages devoted to the general areas of interest is revealing. The general section (Part I) devotes 70 pages to introductory material, 26 to pedology, 116 to ecology, 69 to applied aspects (including the effects of soil pesticides and cultural practices on populations), and 27 to zoophagous fungi. The methods section (Part II) includes 90 pages devoted to sampling and estimating (including arthropod, annelid, nematode and general), 14 on culturing, 25 on preparations, and 10 on physiology.

This book should prove particularly useful to all entomologists, zoologists, nematologists, agronomists, microbiologists, plant pathologists, and others concerned with animal life in the soil. Established workers will find it of real value for the complete and critical way it brings together a difficult and widely scattered literature. Younger investigators will appreciate both the literature coverage and the clear and concise consideration of appropriate equipment, techniques and procedures. The fact that a long list of contributors (almost all from the British Commonwealth and the Continent) collaborated with a competent and critical editor makes the book more comprehensive but perhaps less well organized than any one person might have written. The 171 figures include 157 well-executed line drawings and 14 halftone photographs. This book is well printed on good quality paper, attractively bound, and remarkably free of typographical errors. Soil Zoology is a reference work, rather than a textbook, and an excellent one in an important area that has not heretofore been similarly treated. I rate it a classic.

JOHN H. LILLY

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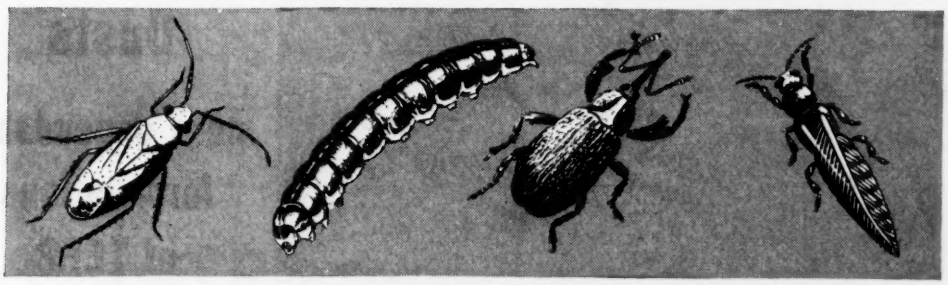
TOXAPHENE IS RECOMMENDED FOR



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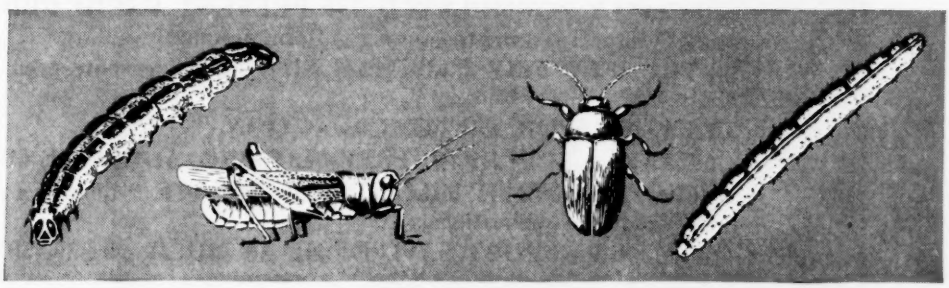
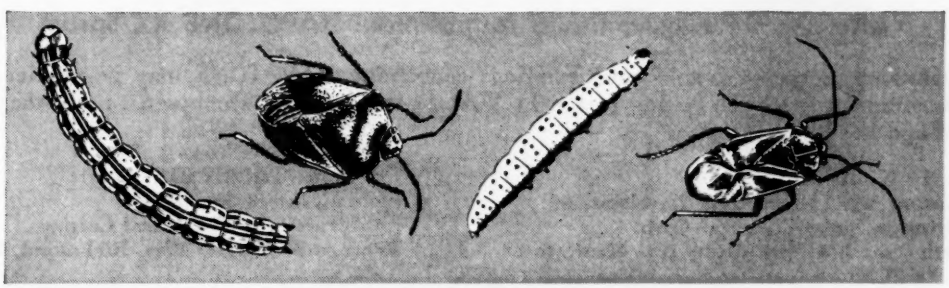


OF MORE THAN 200 INSECT PESTS



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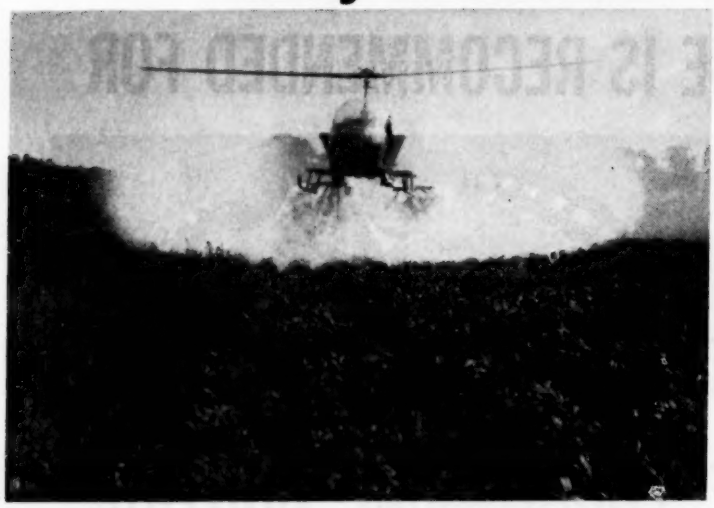

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Also available as a liquid formulation: VAPOTONE XX Spray

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California Spray-Chemical Corp.
North Church & New Albany Rd., Moorestown, N.J.

Dr. R. K. THOMPSON
Field Research Supervisor
California Spray-Chemical Corp.
Lucas and ORTHO Way, Richmond, Calif.

On all chemicals, read directions and cautions before use

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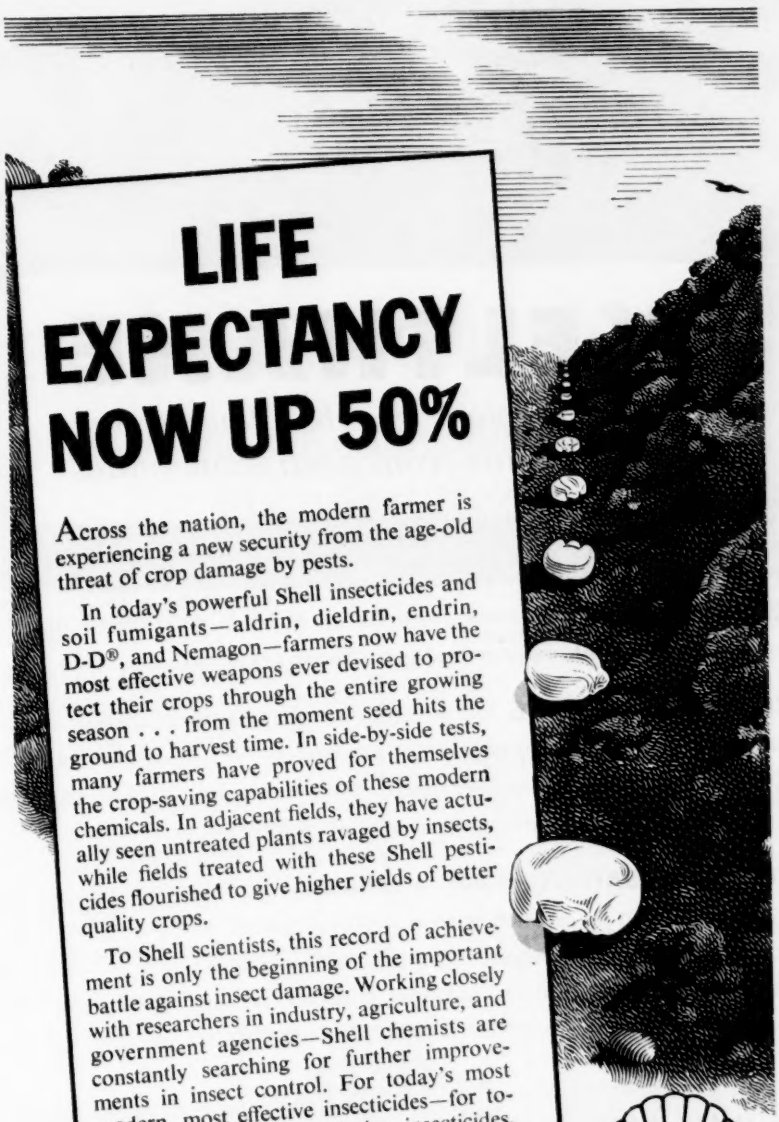

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In today's powerful Shell insecticides and soil fumigants—aldrin, dieldrin, endrin, D-D®, and Nemagon—farmers now have the most effective weapons ever devised to protect their crops through the entire growing season . . . from the moment seed hits the ground to harvest time. In side-by-side tests, many farmers have proved for themselves the crop-saving capabilities of these modern chemicals. In adjacent fields, they have actually seen untreated plants ravaged by insects, while fields treated with these Shell pesticides flourished to give higher yields of better quality crops.

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KELTHANE, the new miticide from Rohm & Haas, now offers improved mite control of both nursery and greenhouse plantings.

KELTHANE IS EFFECTIVE. Even mites resistant to present insecticides and fumigants can now be effectively controlled with this new miticide. KELTHANE controls red spider mites, cyclamen mites, and several other species which infest roses and ornamental plants.

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KELTHANE IS EASY TO USE. It mixes readily with water and forms stable emulsions. Its long-lasting residual action cuts down applications—one spraying lasts for several weeks. Commonly used insecticides and fungicides can be added to the same spray.

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Pyrenone (technical *piperonyl butoxide* and *pyrethrins*), rotenone and pyrethrum are exempt from the requirements of a tolerance under the Miller Bill. They may be used right up to the time of harvest.

Pyrenone Wheat Protectant, Pyrenone Grain Protectant and Pyrenone liquid protectants can be applied to wheat, corn (including popcorn), buckwheat, barley, rye and rough rice, in accordance with package directions, for protection during a normal period of storage with a *single* application.

UNDER THE MILLER BILL *piperonyl butoxide* has an approved tolerance of 20 p. p. m. and *pyrethrins* a tolerance of 3 p. p. m. These tolerances are in *excess* of the quantities specified for use in the package directions.

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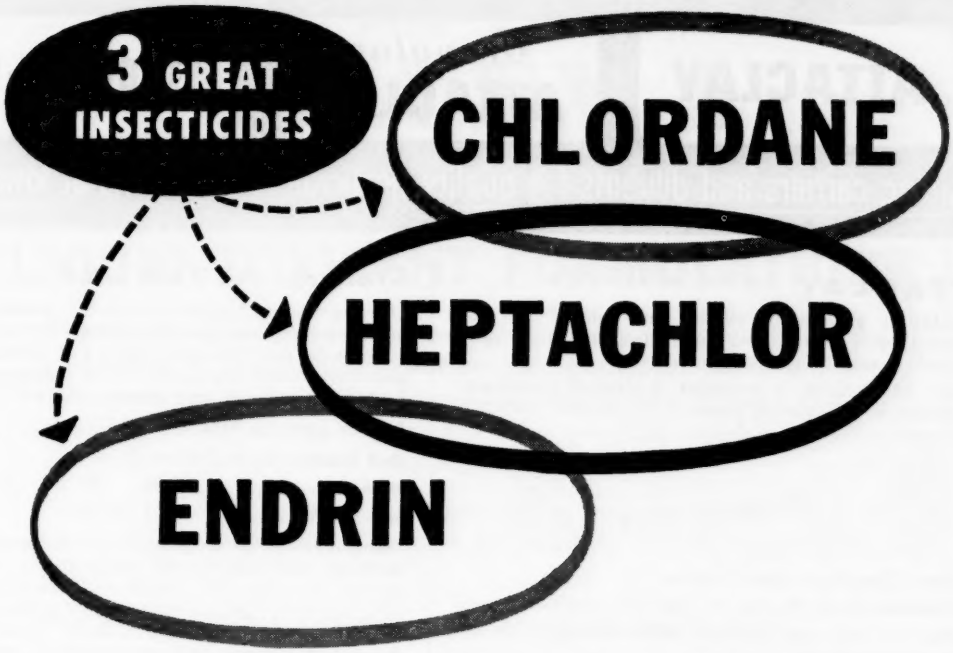

CONTROL THESE INSECTS

CHLORDANE: Ants, Armyworms, Blister Beetles, Boxelder Bug, Brown Dog Tick, Cabbage Maggot, Carpet Beetles, Cattle Lice, Chiggers, Cockroaches, Crickets, Cutworms, Darkling Beetles, Dog Mange, Earwigs, Fleas, Flies, Grasshoppers, Household Spiders, Japanese Beetle Larvae, Lawn Moths, Lygus Bugs, Mole Crickets, Mosquitoes, Onion Maggot, Onion Thrips, Plum Curculio, Sarcoptic Mange, Seed Corn Maggot, Sheep Ked, Silverfish, Sod Webworms, Southern Corn Rootworm, Strawberry Crown Borer, Strawberry Root Weevils, Sweet Clover Weevil, Tarnished Plant Bug, Termites, Ticks, Wasps, White Grubs, Wireworms...and many others.

HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Asiatic Garden Beetle Larvae, Black Vine Weevil, Root Maggots, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevil, Cotton Fleahopper, Cotton Thrips, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, False Wireworms, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrips, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Spittlebug, Strawberry Root Weevils, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms ...and many others.

ENDRIN: Budworms, Cabbage Worms, Cotton Boll Weevil, Cotton Bollworm, Cotton Fleahopper, Fall Armyworm, Grasshoppers, Hornworms, Leafworms, Rapid Plant Bug, Spiny Bollworm, Sugar Beet Webworm, Tarnished Plant Bug, Thrips.

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Attaclay is a versatile carrier and diluent for the formulation of insecticides, fungicides, and other agricultural chemical dusts and powders.

Source—Attapulgite, a complex hydrated aluminum magnesium silicate—Chemical Analysis (volatile free basis)

SiO_2	67.0%
Al_2O_3	12.5%
MgO	11.0%
Fe_2O_3	4.0%
CaO	2.5%
Other	3.0%

Hardness—Degritted, non-abrasive.

Bulk Density—27-31 lbs./cu. ft. (packed condition).

Fineness—1-2 microns (surface mean diameter, by air permeation method).

pH—Neutral—no effect on acid- or alkali-sensitive materials.

Sorptive Capacity—following table illustrates Attaclay's unique sorptive properties:

Toxicant	Wt. % in Formula	Toxicant	Wt. % in Formula
Chlordane	40 and 50	Parathion	15 and 25
DDT	50 and 75	Aldrin	20 and 25
DDD (TDE)	50	Dieldrin	50
EPN	25	Endrin	25
Toxaphene	40	Dilan	25
Benzene Hexachloride	50, 75 and 85 depending on gamma content	Methoxychlor	50
		Heptachlor	25
Lindane	25	Aramite	25

Wettability, Suspensibility—Wets very rapidly, disperses readily.

Flowability—Accepts unusually large amounts of toxicants yet stays lump-free. Flows loosely during and after formulation and after long periods of storage, even under adverse conditions.

Compatibility—Physical and chemical compatibility is well established.

Dustability, Conditioning—Imparts lightness and fluffiness to dust base; adjusts volume-weight of finished dusts; conditions formulas for improved flowability and covering power.

Uses—*Grinding aid* for pesticide chemicals; *Carrier* for dust bases, wettable powders, impregnation formulas; *Diluent* for field strength dusts; *Conditioner* for finished formulas, fertilizers, and other chemicals such as ammonium sulfate.

ATTACLAY X-250

Recommended as a carrier and conditioner in those applications in which the superior sorptive capacity and fineness of Attaclay are not necessarily required. Attaclay X-250 is 96-97% finer than 200 Mesh and 100% finer than 100 Mesh, is 10-15% less sorptive than Attaclay, and has a bulk density (packed condition) of 34-36 lbs./cu. ft.

Granular ATTACLAY

Development of Granular Attaclay, based on our years of experience with granular grades, opens new opportunities for pesticide processors. For Granular Attaclay provides a most efficient means of formulating granular pesticides and applying them to the soil and to water.

Source—same as Attaclay.

Bulk Density—32-34 lbs./cu. ft.

Mesh Sizes—8/15 15/30 20/30 30/40 30/60

pH—see Attaclay.

Adsorptivity—Capacity based on laboratory kerosene sorption and commercial preparations (depending on grade): 36% to 42% Aldrin; 40% to 46% Chlordane; 33% to 39% Dilan; 40% to 46% Toxaphene; 33% to 38% Parathion. (DDT, BHC, lindane, dieldrin depend on density and concentration of solution used for impregnation.) This high sorptive capacity of Granular Attaclay permits formulation of high concentrates of toxicants without danger of caking in bags or hopper.

Formulation—Mixes well in either ribbon or rotating drum type mixer. Simple alterations to ribbon mixer practically eliminate particle breakdown. Little, if any, breakdown is encountered in drum type mixers.

Application—Shows greatest superiority over dust and emulsion formulations when applied from the air. Distribution pattern extremely uniform. Ideal for mixing granular pesticide concentrates with fertilizer to eliminate segregation. Gives excellent distribution of toxicant in soil or water. Can be used in broadcast, band or row treatment with conventional equipment.

Toxicant Release—Can be controlled by various methods. "AA" RVM Granular Attaclay disintegrates in water and tends to release toxicants faster than does the "A" LVM Granular Attaclay. Indications are that specific surfactants can control toxicant release.

Compatibility—Well established through commercial formulations.

Uses—Carrier for liquid impregnations; Soil conditioner; Anti-caking agent.

Put our agricultural chemical products to your most exacting tests. Write for generous samples and technical assistance.

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This highly adsorbent carrier has been developed especially for the processing of impregnated concentrates, both dust bases and wettable powders. Diluex A has found wide acceptance in many new insecticide developments requiring a base material capable of effectively carrying liquid and waxy organic toxicants.

Ease of handling, increased output, and an improved uniformity of final product commend Diluex A in grinding and blending operations.

Samples and additional data will be forwarded upon request.

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Adsorptive FLOREX granules offer a superior base for granular soil pesticides, mosquito control formulations and insecticide-fertilizer additives. Available in standard meshes 30/40, 30/60, and 16/30. Special meshes tailored for experimental formulas.

Properties of DILUEX A

Color: Light grey to cream

Composition: Aluminum magnesium silicate (Florida Fullers Earth)

Bulk: 19 to 21 lbs. per cubic foot, loose
33 to 36 lbs. per cubic foot, packed

Mesh: 90 to 95% finer than U.S. 325

Specific Gravity: 2.2 to 2.4

Surface Mean Diameter: 1.5 to 1.7 microns

pH: 6.8 to 7.1 (AOAC method)

Abrasiveness: Lowest rating

Adsorption: Adequate for impregnating liquid toxicants at recommended concentrations

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with interchangeable orifice tips

Supplied with male or female connection, in choice of over 400 interchangeable orifice tips. Give full range selection to meet any requirement. Flat spray, hollow cone, full cone and straight stream types. For all liquids including those with suspended solids. For use with boom or gun type sprayers.

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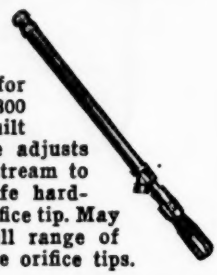
For use at any pressure up to 250 pounds. Light in weight and easily handled. Fits comfortably in hand. Positive shut-off valve with lock. For use with all TeeJet interchangeable orifice tips. Supplied with or without large capacity strainer in handle.

GunJet Spray Guns



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FOR INFORMATION . . . write for general catalog No. 30 . . . Bulletins 66 and 71, BoomJet Spray Nozzles . . . Bulletins 65, 69 and 80, GunJet Spray Guns . . . and Bulletin No. 68, Trigger TeeJet Shut-Off Valves.

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Availability: Trithion is suggested for field evaluation in both spray and dust applications. Samples are available for experimental purposes as a liquid concentrate containing 4 pounds of technical Trithion per gallon, a wettable powder containing 25% by weight; and as dusts in 1 to 5% concentrations.

Information: Additional technical information may be obtained from the Agricultural Research Laboratory of the Stauffer Chemical Company, 1496 East Fremont Avenue, Mountain View, California, or Stauffer Chemical Company, Research Center, Chauncey, New York.

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Pound for pound, Celite gives you far greater performance than other inerts since only ten pounds give you a full cubic foot of extender. Celite gives you far greater value since it absorbs up to 50% more poison than other diluents on a dollar-for-dollar basis.

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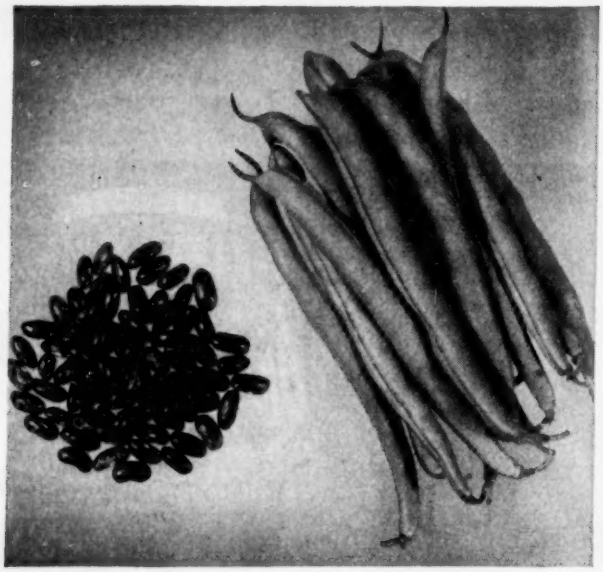

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Aramite, today's safest strongest mite-killer, promises growers bigger, more profitable yields of better fruits, vegetables, row crops, cotton and ornamentals. This is possible because Aramite can effectively control a wide variety of mites at low cost per acre without harming humans, animals or crops. Further advantages of Aramite are ease of use, long residual effect, high compatibility and harmlessness to natural predators.

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by

J. G. NEEDHAM and P. W. CLAASSEN

A supply of this supposedly out-of-print monograph has been located. The book contains 397 pages which includes 50 plates of black and white illustrations and an index. It was published in 1925. Price $5.00 in North America and U. S. possessions, $5.50 elsewhere.

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